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Mr Benn's mixture of good and bad

BRITISH industry is in a woeful mess; there is little disputing that and small comfort in the knowledge that nowhere in the world is industry doing outstandingly well at present. Nor is the malaise a thing of the recent past. For many years the danger signals have been flying, as Britain's growth has become sluggish and as industrial investment has fallen off.

A long period of decline will require a long period of recovery; certainly no tactical change of course that a single budget could effect will help much and this is the importance of Mr Benn's proposals in *The Regeneration of British Industry*, just published as a White Paper (Cmnd 5710). Nothing less than an immense strategic rethink is necessary and the time when industrialists could simply ask to be left alone is well past. The crudity, so far, of the advertisements of Aims of Industry—a group of employers dedicated to free enterprise—suggests that there are many who still do not appreciate that what is desperately needed now is discussion and not rhetoric. Lack of business confidence, which is what the decline in investment is all about, cannot be treated by urging support of the status quo.

That, at least, is certainly far from Mr Benn's intention. Government and industry, the document argues, have dealt too remotely with each other; and industry has seen the government's function as one of regulation. Now it is time for partnership, and this is being proposed in two fairly distinct ways.

First, there will be Planning Agreements with major firms to ensure harmony with national needs and objectives and to provide a better basis for governmental aid. The agreement will be a rolling three-year look at company plans, and if necessary will provide discretionary financial assistance. The agreement will be drawn up by management 'in close consultation with trade union representatives' who will be provided with all necessary information for effective consultation. The government sees this as a major advance in industrial democracy. In return for companies opening up their plans to Whitehall, they will have the benefit of 'the government's views on the likely development of the economy'.

The second proposal is for a National Enterprise Board (NEB) and it is through this that the government will channel large scale investment in exchange for equity. It will also take over the government's holdings in companies such as Rolls-Royce (1971) Ltd, assist industrial reorganisation, start new ventures and extend public ownership into profitable manufacturing industry by buying up to 100% of the equity. Criteria for acquiring a profitable company will include the danger of its passing into "unacceptable" foreign control, and the

stimulation of competition. The NEB may also take over an ailing company for reasons of regional employment or "industrial policy". The board will ensure that enterprises under its control fully involve employees in decision making. Mr Benn is restrained by the requirement that all major deals require government approval and the Cabinet, much less to the left on average than Mr Benn, will presumably moderate his undoubted vigour.

The timing of the document makes it quite obviously an election manifesto since nobody seriously believes there will not be an election before the White Paper can be put to the vote. And it is this reminder of the fluctuations of politics which casts some doubts on the two schemes. If a Labour government guarantees a three-year planning agreement, will a subsequent Conservative government dismantle it? Surely this would provide for even less investment confidence and so there is a need for the two major parties to hammer out something which they can each live with. The document, as it now exists is more moderate than was originally planned. Even so, the thought of trade unions looking at the books and helping to make decisions will still terrify most Conservative Members of Parliament and may make the whole programme unacceptable if Labour is still a minority government when legislation is being considered.

This would be most unfortunate. If there is one thing that desperately needs attention in Britain, it is the them-and-us divide which permeates industry and most other things too. Industrial democracy, common in many other countries, has to come soon and Mr Benn is right to push for it.

The other compulsory match for industrial management is with government, and this is a dubious affair indeed. The problem is that government has no evidence to show that it has any unusual skills or knowledge to offer. No doubt there will be some bright people recruited into the NEB and Planning Agreement section of the Department of Industry but on the whole staffing will be accomplished by shuffling the pack of civil servants. Is there any evidence that they will be a desirable commodity for industry? And the political masters; well they are much the same as those who were going to harness the technological revolution in 1964, and it is the failure to do that which has contributed much to Britain's present depressed state. No doubt the agreements will allow civil servants to pass on the minister's thinking in their offices instead of their clubs, but vigorous industry is more likely to be able to shape the minister's thinking by its successes than to be shaped itself because of its failures.

If the planning agreement concept suggests the gaining of doubtful benefits from Whitehall, the NEB investment and takeover scheme is worse. The reward for running a profitable company is to find not only the unions but also the government wanting a large piece of the action. No self-respecting manager will stay with a firm in which success is recompensed by loss of responsibility. If there is so much talent in Whitehall, it should surely be used to revive less successful industry.



The changing relationship between science and technology

J. Langrish of the Manchester Business School examines the way in which the relationship between science and technology has changed in the past and is about to change again.

In recent years, the belief that technological change is somehow based on scientific advance has been increasingly challenged. Price¹, for example, has claimed: "The naive picture of technology as applied science simply will not fit the facts. Inventions do not hang like fruits on a scientific tree. In those parts of the history of technology where one feels some confidence, it is quite apparent that most technological advances derive immediately from those that precede them".

Technological change has been increasingly seen as the adaption of existing technological concepts in response to demand. Schmookler², for example, has attempted to show that the variation in inventive activity between different American industries is explicable in terms of the variation in demand, concluding that economic growth determines the rate of inventive activity rather than the reverse. None the less, governments have continued to invest in scientific research at a level much greater than other activities which can also claim cultural and prestige benefits. Science receives much more money than the Arts because it is believed to be useful. When science is not used, it is claimed that there is a communication barrier; the alternative possibility that it is intrinsically of no use is not examined.

One of the major problems in looking at the usefulness of science is a matter of definitions. What is Science? What is Technology? To avoid this problem, we must ask questions such as, what is the effect of university research and research in government laboratories on industrial change, or on improvements in health and agriculture.

A study³ of 84 British technological innovations showed that "demand pull" occurred more frequently than "discovery push" and that out of 158 key technical ideas made use of in innovations, 56 originated within the innovating firms and of the remaining 102, 7 came from a British university and 17 from a British government labora-

tory (including Research Associations).

The apparent conclusion that ideas from British universities had little effect on British innovation was challenged in a variety of ways. One of these was the claim that although industrial innovation may be based on industrial research, the day to day progress of industrial research depended on university research in ways that would not be revealed in looking at 'key ideas'. It was pointed out, for example, that industrial chemists spend time reading the results of university chemical research.

To examine the relative contribution of university research in the literature cited by industrial researchers, seven review articles written by British industrial chemists were examined⁴. The reviews contained 567 references to other publications (including patents) and the institutional origins of 396 of them were identified. Only 23 out of the 396 references were to the work of British universities and of these, 7 stated that some industrial finance had been involved in the research. British government institutions seemed to be more interesting to the industrial reviewers in that 45 of the references were involved. Table 1 compares the relative importance of the institutional origins of the 102 "key ideas" with the origins of the 396 references and also the origins of 452 abstracts to be discussed later.

These results suggest that British university research has little impact on British industry. Why then does the British government continue to finance university research? One possible reason is that university research only occasionally produces results of economic benefit but when it does the benefits are so large that they outweigh the total costs of all university research for several decades. An example of this kind in the present century would be the research that led to atomic power. A more fruitful source of such examples is the last half of the last century.

There is little doubt that German university research in organic chemistry, for example, provided the foundations for the German synthetic dyestuffs industry and subsequent advances in pharmaceuticals, synthetic rubber and plastics. Similarly the electrical industries can be seen as being based on nineteenth century academic research.

At the time of the First World War Britain realised that Germany had advanced industrially by using the results of scientific research, and in 1917 the Department of Scientific and Industrial Research was established. Since then, the British government has ploughed increasing amounts of money into scientific research in the belief that what was good for Germany in the last century should be good for Britain now.

But times have changed. Reliance on the German example does not seem to have been justified. One possible explanation is that the relationship between science (university research) and technology (industrial practice) has changed since the last century. To test this hypothesis, abstracts produced by the *Journal of the Society of Chemical Industry* were examined. For some eighty years, this Journal produced abstracts of the world's literature that might be of relevance to industrial chemists. Even in the last century, large teams of abstractors were used, reducing the possible bias of an individual. The abstracts were classified according to industrial subject matter, and those classes which might be considered to rest on organic chemistry were chosen for examination.

The original publications selected by the abstractors as being worth the attention of industrial chemists were consulted and wherever possible, institutional origins identified. Initially, the years 1884, 1917, 1935 and 1952 were examined but the difference in institutional origins between 1884 and 1917 was so great that the year 1899 was added to the investigation.

Table 2 shows the institutional sources of abstracts in randomly selected editions of the Journal for the given years. (A small proportion of abstracts in all years defied attempts to identify origins. The figures given are for identified sources.)

Teaching establishments are included under the category of university as are research laboratories and institutions attached to universities. The 'government' category includes Research Associations, government-financed industrial laboratories and military establishments.

Several of the abstracts were to patents some of which caused difficulties in the assignment of origins. Early patents usually give the name and pro-

fession of the inventor. In most cases the profession enabled a classification to be made, such as 'professor'—university; 'merchant'—industry. But descriptions such as 'gentleman' or 'subject of the Czar' had to remain unclassified unless the inventor could be identified from other sources. Papers by joint authors from different types of institutions were scored $\frac{1}{2}$ each.

It can be seen from Table 2 that there has been a marked change in the relative importance of the institutional origins from European university (mainly German) to European industry and then to American industry as being the major contributors selected by the abstractors.

Although some of the change may be due to changes in abstractors or journal policy, the large decline in the relative importance of university publications from 61% in 1884 to 6% in 1952 and the steady growth in the importance of American industry from 0 in 1884 to 54% in 1953 can be assumed to be the result of a change in the relationship between university research in organic chemistry and those industries which use organic chemicals.

The data for 1952 are included in Table 1 for comparison with the studies previously mentioned. The data are based on an examination of 544 abstracts of which 59 could not be found, 6 had insufficient information and 27 were unclassifiable patents, leaving 452 identified origins. The three different studies give different percentage inputs but it should be noted that in all three studies:

- Foreign industry was the major source of inputs

- UK industry was the second most frequent source in two studies and the fourth most important in the other

- UK university was either the least important source or next to the least.

The dramatic change which has taken place since the days when German university research was providing results of direct relevance to the German dyestuffs industry may be explained by two alternative hypotheses:

(1) Industry has taken over its own research. The massive growth in industrial research since German industry employed teams of chemists to search for new dyes may explain the decline in apparent relevance of university research. However, university research has

Table 1 Institutional origins of literature cited in British industrial research

Origins	% of 102 ideas	% of 396 references	% of 452 abstracts (1952)
UK Industry	22	10	19
UK University	7	6	1
UK Government	19*	11	1
Foreign industry	40	40	68
Foreign university	3	21	5
Foreign government	9*	12	6

*Government and military combined

Table 2 Change with time of institutional sources of abstracts in *Journal of Society of Chemical Industry* for industrial areas connected with organic chemistry

Year of journal	1884	1889	1917	1935	1952
Institutional source	%	%	%	%	%
US industry	—	4.8	14.5	38.1	53.8
UK industry	8.8	17.3	6.2	9.3	19.0
European industry	23.5	44.7	52.4	24.1	13.6
Other industry	—	1.0	0.7	0.5	0.9
Total industry	32.3	67.8	73.8	72.0	87.4
US government	—	1.0	1.4	1.9	3.6
UK government	—	1.0	—	0.5	1.1
European government	5.9	1.9	2.1	3.4	1.3
Other government	—	1.0	2.1	0.5	1.1
Total government	5.9	4.8	5.5	6.3	7.2
US university	3.0	1.0	1.4	1.9	2.6
UK university	—	7.7	6.2	0.8	0.7
European university	58.8	18.7	13.1	15.3	0.2
Other university	—	—	—	3.7	2.0
Total university	61.8	27.4	20.7	21.7	5.5

Table 3 Change with time of institutional source of publications referred to by British industrial chemists in reviews of 'polyolefin' chemistry

Date of review	1957 (n=28)	1961 (n=71)	1967 (n=102)
Institutional source	%	%	%
UK industry	3.5	12.0	12.0
US industry	41.5	46.0	69.5
European industry	5.0	7.0	
Total industry	50.0	65.5	81.0
UK university	—	—	2.0
US university	7.0	—	12.0
European university	43.0	28.0	
Total university	50.0	28.0	14.0
UK industry-university collaboration	—	—	3.0
US industry-university collaboration	—	6.5	2.0
European industry-university collaboration	—	—	
Total industry-university collaboration	—	6.5	5.0

also grown enormously since the last century. (An interesting account⁷ of the growth of British industrial research has been given by Sanderson.)

(2) A new branch of science is only useful in its early days. The early days of astronomy as a science were linked with economically important attempts at improving navigation but astronomy has hardly been useful since then. (Even earlier, astronomy was used to maintain the power of priestly castes.) The early days of atomic power involved theoretical physicists; since then the theoretical physicists and the designers of nuclear power stations have moved further apart.

The early days of organic chemistry involved the production of new chemical substances and new ways of making them. Since then the university chemist has been more concerned with understanding the results of early research; the industrial chemist has been concerned with using the early

knowledge and is not too interested in later theoretical developments.

One of the seven review articles referred to earlier was about 'polyolefins' and only 14 out of 102 identified references were to university research in spite of the fact that the present industrial importance of such materials as polypropylene started with the non-industrial research of Ziegler and Natta who may be considered to have founded if not a new branch of science, at least a new stem entitled stereo-specific polymerisation.

It was decided, therefore, to test the above hypothesis by looking at earlier reviews of 'polyolefins' by industrial chemists. Table 3 shows that the relative importance of university contributions dropped from 50% in 1957 to 14% in 1967, although the absolute number of university contributions remained constant.

Another point of interest is that British industry began publishing items of interest to the reviewer before British universities. This was not because British universities ignored stereo-

specific polymerisation. It was because universities concentrated on the scientific problem of describing and explaining the mechanisms involved and the structure of the polymers produced whilst industry concentrated on obtaining manufacturing processes and improving properties such as light resistance. From a common origin, the discovery of stereospecific polymerisation, academics and industrialists have moved in different directions.

The relationship between university research and industry may well be a function of the degree of development of the area concerned. Once a new area has been established, the aim of science is to understand; the aim of technology is to make it work, and industry has been very successful at making things work without too much reliance on understanding.

Industry makes use of the trained manpower supplied by universities. It also uses new techniques such as chromatography, developed in universities. But the new products and processes of industry seem to depend on a combination of existing technological concepts, economic pressures and empirical research with scientific understanding not being very relevant.

This does not mean that university research is a waste of money; the situa-

tion may be changing. Concern about such matters as resource depletion, ecology, pollution, fire risk, health hazards and quality of working life coupled with the increasing scale of industrial operations may mean that industry will have to pay more attention to understanding what it is doing.

In the study of 84 innovations mentioned earlier, the only example of theoretical work in a British university assisting British industry was in the area of steel frames for building construction. A concern for human safety together with a shortage of steel led to the use of Baker's theory of plastic flow to design buildings which are safe but use less steel.

It could be that in the future, many sections of industry are going to require an increasing reliance upon theoretical research aimed at understanding, as the empirical approach, which has been so successful, joins the quest for economic growth as a thing of the past.

It would seem, therefore, that there are both macro and micro effects leading to changes in the relationship between science and technology.

At the micro level, the relevance of a particular part of science has depended on its degree of development. At the macro level, the relationship

seems to move in cycles, with the first half of the nineteenth century involving little reliance upon scientific theory (although the scientific technique of methodical observation was made use of) the second half of the nineteenth century being characterised by a series of inputs from research concerned with exploring scientific problems, the first half of the present century being a triumph of industrial methodical empiricism and the future requiring an increasing dependence on understanding.

I thank the Department of Education and Science and the Programmes Analysis Unit for funding parts of this research and Trevor Parkinson for his considerable ingenuity in tracking down the institutional origins of some of the more obscure references.

¹ de Solla Price, D. J., in *Factors in the Transfer of Technology* (edit. by Gruber and Marquis), (Massachusetts Institute of Technology, 1969).

² Schmookler, J., *Invention and Economic Growth* (Harvard University Press, 1966).

³ Langrish, J., Gibbons, M., Evans, W. G., and Jevons, F. R., *Wealth from Knowledge* (Macmillan, London, 1972).

⁴ Langrish, J., *Chemistry in Britain*, 8, 330 (1972).

⁵ Sanderson, M., *Science Studies*, 2, 107 (1972).

international news

A WORKING party under the chairmanship of Lord Ashby, Master of Clare College, Cambridge is being set up by the Advisory Board for the Research Councils to "make an authoritative assessment of the potential benefits and potential hazards of techniques which allow the experimental manipulation of the genetic composition of microorganisms".

At the same time the Medical Research Council (MRC) has made public the fact that they have asked their staff members not to embark on experiments in bacterial manipulation vetoed in the recent National Academy of Sciences (NAS) statement.

The letter circulated to directors of those MRC units which have the capacity to carry out work in this field asked that no experiments of type I and II in the NAS statement be undertaken in the near future until the working party has reported. This covers work on recombinant drug resistance plasmids that do not occur

Improper plasmids?

by Eleanor Lawrence

naturally, and the linking of animal virus DNA to plasmid DNA and its subsequent introduction and replication in a bacterial cell.

Directors were also asked not to initiate research on linking animal DNA to plasmid or bacterial DNA, which, according to the NAS statement, "should not be undertaken lightly." Experiments of this kind have already been carried out in the United States.

According to a spokesman for the MRC, the council supports no programmes actually involving research of the kind mentioned by the NAS, although the separate techniques are being studied in various laboratories.

He estimated that about 50 laboratories in Britain have the capacity to carry out genetic manipulation of the sort vetoed by NAS, but that until now there had been a self-imposed holding back from this type of experiment, which has been technically possible for some time.

The establishment of a working party to clarify the issue is generally welcomed, as the part of the NAS statement referring to drug resistance plasmids is felt by some to be ambiguous; it could, for example, be interpreted as banning experiments necessary for important public health work. Other workers also feel that the ban on introducing animal genes into bacterial cells could hold up work on new ways of producing important substances such as insulin.

The working party will consist of "high-level scientists" and the Advisory Board for the Research Councils hopes to be able to announce the membership within the next two weeks. □

Focus on astronomy

by John Gribbin and Ian Ridpath

A RECENT flurry of activity related to observational astronomy has emphasised that the obituaries for British optical astronomy may have been written too soon. Certainly the prospects for optical astronomy in Britain are dim; but the stirrings of the supposed corpse are having repercussions around the world, emphasising the international nature of modern astronomy.

Anglo-Australian Telescope: The AAT is the furthest of these projects from Britain geographically, but perhaps the most important in scientific terms. In spite of the usual difficulties of marking out territory on international projects (which will be familiar to readers of Fred and Geoffrey Hoyle's book *The Inferno* (Heinemann, 1973)) the project is still more or less on schedule. The official timetable, which sees commissioning of the telescope in October with completion of alignment and testing of optics and instrumentation in December of this year, looks a trifle optimistic. But it does seem that the facility will be ready for use early in 1975.

Infrared telescope for Hawaii: The SRC recently announced the decision to go ahead with construction of a 3.8 m (152-inch) infrared flux collector on Mauna Kea in Hawaii. This ends long speculation about the future of British infrared astronomy, and will result in the world's biggest 'purpose built' infrared telescope being located at a site which is recognised by astronomers as one of the best in the world. The SRC will lease the site for the instrument from the University of Hawaii, which already operates an observatory on Mauna Kea, at a height of 4,200 m.

This project has suffered more than most from political intervention, with sites such as Tenerife being ruled out because of poor relations between the British and Spanish governments. But for once the astronomers may have come out well from the politicking, since it seems likely that the Hawaii site would have been felt too remote for administrative convenience if an alternative closer to home had remained viable. The site is also convenient from a practical point of view, because it is far enough south to provide a good view of the galactic centre, a region of great interest to infrared astronomers. The construction costs for this telescope are estimated as £1.25 million at January 1974 prices, and construction is expected to take three years.

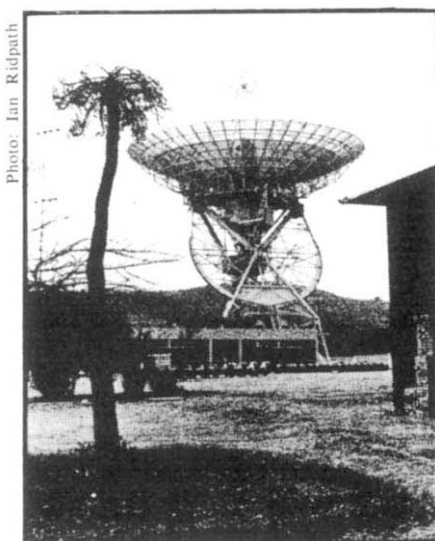
Mr Gordon Carpenter, the project manager, said last week that tenders

are now out and are expected back in early October. The site allocated is on the same mountain ridge as the University of Hawaii 88-inch optical telescope, and so most of the access roads and so on are already there. It should be possible to pour the first concrete in 1975, he said.

All this must make hot favourite as the location for the Northern Hemisphere Observatory, about which a decision is thought to be imminent.

South Africa: Amidst all this lightening of the astronomical gloom which has been so prevalent in recent years, a few storm clouds remain as a reminder that astronomers have not entirely abandoned their centuries-old tradition of internecine squabbling.

Since the opening of the new South



Hartebeesthoek tracking station

African Astronomical Observatory, with its observing station at Sutherland in the Karoo and its headquarters at the Cape Observatory, the former Republic Observatory in Johannesburg has been effectively abandoned. The observatory's buildings in Gill Street stand silent and empty, with the 26½-inch refractor, responsible for the observatory's world-renowned work on double stars and classic colour photography of Mars, now under mothballs in its dome.

Only one astronomer remains at the observatory, examining plates taken for minor planets with the 10-inch Franklin Adams telescope at Hartebeestpoort, 50 miles outside Johannesburg.

The decision to cannibalise the Republic Observatory and concentrate efforts at the Sutherland was received with mixed feelings by astronomers a few years ago, and Dr William Finsen, formerly Director of what was once South Africa's national observatory, was among the critics who spoke out against what he saw as a wrong decision by the SRC and the South African

Council for Scientific and Industrial Research (see *Nature*, 229, 355; 1974). Today, Finsen is in retirement and virtually an outcast from the South African astronomical establishment. For good or bad, however, the reorganisation of observational astronomy in South Africa is now essentially complete, and under the forceful direction of the former Astronomer Royal Sir Richard Woolley, it is hardly likely to become a backwater.

South African radio astronomers, meanwhile, like their infrared colleagues in Britain, seem to have benefited from a partially politically oriented decision. The 26-m (85-foot) radio dish of the former NASA deep-space tracking station at Hartebeesthoek, near Pretoria, ceased operation at the end of June this year, officially as part of "an overall consolidation activity" to prune the NASA tracking network. Privately, however, astronomers say that pressure from black politicians in the United States is believed to have influenced the decision. The associated tracking station for Earth-orbiting satellites is to close next year.

The deep-space station, opened in 1960, has been used to track all NASA's unmanned probes to the Moon and planets; it has now been taken over by South Africa's Council for Scientific and Industrial Research, and is being modified to work as a radio astronomy observatory. Initially the aerial will work at a wavelength of 13 cm, and eventually wavelengths as short as 4 cm will be available. The South African radio astronomers hope to have the telescope operational by the end of the year, and perhaps as soon as September. Surprisingly, however, no firm programme has yet been decided; but the interest of the observatory's director, George Nicholson, in quasars and extragalactic work is certain to make itself felt.

Leaving aside this somewhat fortuitous windfall to the radio astronomers, the revival of British and associated optical astronomy can in retrospect be traced back over several years. Certainly five years ago there was cause for concern; but with hindsight it is becoming clear that the problems have been recognised and to a large extent tackled. Projects such as the Schmidt at Siding Spring, the AAT and the new infrared telescope are now becoming physical realities, and allowing for the inevitable delay in the filtering of the resulting benefits through the system, the patient may not only be able to take up his bed and walk but may even be running around in the first team in another ten years or so.

correspondence

Megalithic alignments

SIR,—In your editorial "Science beyond the fringe" (April 12) you speak of archaeology as "plagued by a series of ideas which have achieved a following particularly among the young". The extreme example you give of this is "people busily poring over Ordnance Survey maps of Britain plotting mythical alignments between ancient monuments and erecting fanciful hypotheses about prehistoric technological civilisations." To this, as you say, Professor Glyn Daniel has objected in his *Antiquity* editorials.

Please allow me, as an author on the subject ridiculed in your editorial, the privilege of a brief comment.

You declare that alignments between ancient monuments are "mythical" and hypotheses about advanced prehistoric civilisations "fanciful", although as editor of a scientific journal you are of course aware that your statement of opinion does not necessarily make them so. It is ironical that the first man to detect significant alignments between megalithic sites was your predecessor, Sir Norman Lockyer, editor of *Nature* for the first 50 years of its existence. In fact, despite the studied neglect with which Lockyer has been treated by archaeologists throughout this century, his main conclusions, that megalithic sites were inter-related and orientated by astronomical considerations, are now generally accepted, even by the editor of *Antiquity*, as the result of recent work by Professor Thom and other astro-archaeologists. The quality of criticism brought against Lockyer and his school is typified by the comment with which O. G. S. Crawford, a former editor of *Antiquity*, dismissed the evidence of A. Watkins relating to the deliberate alignment of ancient sites: that it was "based on a misconception of primitive society." This comment perfectly illustrates the determination of modern archaeologists to prefer the basic historical paradigms they have set up above any evidence that might contradict them.

Personal experience shows that archaeologists, Professor Daniel included, simply refuse to review facts and evidence tending against current orthodoxy. In a book published by Garnstone Press early this year, *The Old Stones of Land's End*, I demonstrated, in a way that must convince any reasonable person who cares to

check the facts, the existence of planned megalithic alignments in West Cornwall. The book attracted a good number of local reviews but not one public comment by any archaeologist. It is therefore rather galling to read your account of archaeologists being "plagued" by theories of the inter-relationship of megalithic sites. Would not their best remedy be to attempt a serious refutation of the evidence offered them?

Your faithfully,

JOHN MICHELL

11, Miles Buildings,
Bath, Somerset, UK

Call for biohazard legislation

SIR—Although Brian Ford (*Nature* August 2) refers to various Acts of Parliament, he makes no mention of the Safety and Health at Work etc. Act which received the Royal Assent on July 31. Clauses 2(2)(b) and 3(1) of the Act place a general duty on employers to protect both their workers and other persons from risks to health in connection with the use, handling, storage and transport of articles and substances; and under Clauses 15 and 16, the Secretary of State may make Regulations, or the Health and Safety Commission may prepare and issue Codes of Practice (or approve Codes of Practice prepared by others) for any of the general purposes of the Act. I would therefore suggest that no further legislation is necessary and that the way is clear for agreed Codes of Practice to be promulgated to deal with the different groups of organisms, as Brian Ford suggests.

Yours faithfully,

J. A. TANNAHILL

Employment Medical Advisory Service,
Department of Employment,
London, UK

Population policy study

SIR,—I should like to bring to the attention of readers of *Nature* a survey of the field of reproductive biology and contraceptive development sponsored by the Ford Foundation. The Foundation has asked for an inventory of where we stand in terms of (1) knowledge concerning the reproductive process and fertility control and (2) the human and financial resources that are being brought to bear on the matter of bringing population growth

within tolerable limits. For the conduct of this study, a headquarters has been established in Boston with a worldwide network of collaborators and consultants and an international advisory committee drawn from public and private donor agencies, universities and the pharmaceutical industry.

The force that motivated this major study is the accumulating evidence that an inadequate global effort is being waged in this field. The illusion is that the pressure of more immediate bio-social problems supersedes the need to control human fertility. The facts are otherwise.

The survey was initiated in July 1973 and is expected to be completed in 1975. The findings will be published in full on completion of the survey and as interim reports on special topics.

The comments, views and opinions by readers of *Nature* concerning any or all aspects of this survey will be welcome and helpful in drafting recommendations and guidelines for building on present progress in the understanding of reproductive phenomena and stabilisation of the global populace on a level commensurate with a high quality of life and human dignity.

Yours faithfully,

ROY O. GREEP

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Astronomical independence

SIR,—I write to correct John Gribbin who, in discussing (*Nature* 250, 538, 1974) the thousandth issue of *The Observatory*, wrongly describes it as the house journal of the Royal Astronomical Society. His mistake is understandable since *The Observatory* does, by long tradition, report the proceedings of the Society's meetings and is circulated to all Fellows of the Society. It is, however, published by 'The Editors of "The Observatory"', as is clearly stated in each issue, and has no official connection with the Society. The Editors, indeed, fiercely maintain their independence.

Our house journal is *The Quarterly Journal of the Royal Astronomical Society*.

Yours faithfully,

JOHN SHAKESHAFT

Royal Astronomical Society,
London

news and views

Giant extragalactic radio sources

MANY of the powerful extragalactic radio sources have dimensions much larger than those of the active galaxies which have supplied the vast energies required. In the case of the brightest radio source for example, Cygnus A, most of the radio emission originates, not in the galaxy associated with the source, but in two symmetrically disposed regions, one on either side of it. The peaks of maximum radio brightness are located about 100 kiloparsecs (equivalent to 3×10^5 light years) from the galaxy, the radius of which is only about 10 kpc. Such objects are the most common type of powerful extragalactic radio source found in source surveys although, as compared with the space density of ordinary galaxies, they are very rare indeed.

Detailed radio studies of the structures of these objects have been made possible by the development of the large aperture synthesis telescopes, first at Cambridge and more recently in The Netherlands (the Westerbork Synthesis Radio Telescope, WSRT). Several hundred of the brighter ones have been mapped with an angular resolution of $23''$ and a smaller number with higher angular resolution, that of the Cambridge 5 km telescope being $2''$ at a wavelength of 6 cm. There is no detailed source model which commands universal acceptance since the radio maps have revealed difficulties with each of the models proposed. All models, however, postulate the presence of magnetic fields within the components to produce the synchrotron radio emission, and of cold intergalactic gas in the vicinity of the sources to account for the complex radio structures observed.

Until now the maximum overall dimensions of these radio sources have been thought to be about 1 Mpc, which is the characteristic scale of clusters of galaxies rather than of galaxies themselves. Many radio sources are known to lie close to the centre of clusters of galaxies and for a while it has looked as if there might be a relationship between these two scales particularly since there is good evidence, from X-ray observations, for the presence of hot diffuse gas in clusters. If this relationship were true, however, it would be disappointing from the cosmological point of view because one might have hoped to use the radio components of the largest double radio sources as probes of the diffuse intercluster gas where most of the mass of the Universe may well reside.

On page 625 of this issue of *Nature*, Willis, Strom and Wilson report very interesting observations made with the WSRT and show that radio sources with physical sizes greater than 1 Mpc do indeed exist: the radio sources 3C236 and DA240 are extended, with total physical sizes, projected on to the celestial sphere, of 5.7 and 2.0 Mpc respectively. Credit for drawing attention to these objects goes to workers at the National Radio Astronomy Observatory (Bridle, Davis, Fomalont and Lequeux, *Astr. J.*, **77**, 405: 1972) who, in a definitive survey of bright radio sources at 1,420 MHz, found them to lie in "confused" regions and were unable to decide whether the radio sources in close proximity on the sky were physically related or simply chance associations of unrelated objects. In the first two cases studied by Willis, Strom and Wilson, the maps

show unambiguously that these sources are in fact associated and form components of extended double radio sources on a scale much larger than has been known before.

These observations are important for cosmologists, cosmic ray physicists and for the student of the physics of extragalactic radio sources. For the cosmologist there is the prospect of probing the intercluster gas and setting direct limits to the pressure of such gas. For the cosmic ray physicist, there is now the direct evidence that clouds of cosmic ray electrons can be ejected beyond the confines of a cluster of galaxies in times much shorter than cosmological time scales.

For the radio source theorist, these objects stimulate new speculations. First, nobody has ever been clear about the ultimate fate of a powerful double radio source. The present observations suggest that in some cases the components expand, preserving the relativistic particles within their lobes, until very late stages in their evolution. Second, the observation of polarised radio emission from the components of DA240 indicates surprisingly low densities of cold matter inside the components. Third, and perhaps most interesting, there is the observation of a compact double radio source associated with the parent galaxy in the case of 3C236. It is usually assumed that these components are moving away from the nucleus of the galaxy in opposite directions. The axis of this compact double is almost perfectly aligned with the axis of the giant double source components. On any theory of the extensive components, the latter must be able to 'remember' for hundreds of millions of years the direction in which to supply energy. Similar phenomena have been known before—in the radio galaxies Centaurus A and 3C66 for example—but 3C236 is probably the clearest case yet. Observations of Cygnus A forced Hargrave and Ryle to conclude that a continuous supply of energy over tens of millions of years is essential if one is to account for the structure of the components, but present observations are evidence that a continuous supply of energy may occur for even longer, in the oldest sources. These new observations, and others in progress at Westerbork, will therefore be seized upon by cosmologists and cosmic ray physicists, and will provide yet another link in the chain of our understanding of the physical evolution of extragalactic radio sources.

M. S. LONGAIR

Organisation of sequences of rRNA

THAT the sequences of both 28S rRNA of the large ribosome subunit and 18S rRNA of the small subunit are contained in a single precursor has been known for some time; for this constitutes one of the rare cases where a synthetic system was defined first with eukaryotic and only later with prokaryotic cells. The size of the precursor varies; in HeLa cells it sediments at 45S, so that only 56% is conserved in the form of mature rRNA sequences, but in *Xenopus* it is smaller (2.7×10^6 daltons instead of the mammalian 4.1×10^6 daltons) and almost 80% is conserved. The processing

pathway is best defined in HeLa cells. By utilising an electrophoretic analysis of nucleolar RNAs, Weinberg and Penman (*J. molec. Biol.*, **47**, 169–178; 1970) found that the 45S primary transcript is cleaved to a 41S molecule, which in turn is split into a 32S and a 20S RNA; the 32S molecule then matures into 28S rRNA and the 20S molecule matures into 18S rRNA. By analysing the oligonucleotides into which these species are broken by ribonuclease, Maden, Salim and Summers (*Nature new Biol.*, **237**, 5–9; 1972) confirmed that the 41S precursor contains both 28S and 18S sequences and that the 32S and 20S precursors respectively contain sequences of only the 28S and 18S rRNA molecules. Because oligonucleotides were identified by their content of labelled methyl groups, these experiments showed also that all the methyl groups added to 45S RNA are located in regions of the molecule destined to become mature rRNAs.

The identification by Seeber and Busch (*J. biol. Chem.*, **246**, 7151–7158; 1971) of common 5' terminal sequences in 45S, 32S and 28S RNAs of Novikoff hepatoma cells showed that the 28S rRNA sequences is located at this end of the precursors which contain it. The elegant analysis of RNA secondary structures reported by Wellauer and Dawid (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 2827–2831; 1973) achieved a more precise location of ribosomal RNA sequences in the precursors. The 45S precursor contains a 5' terminal structure corresponding to 28S rRNA, with the 18S rRNA located within the remaining sequences; the 41S RNA structure is similar but lacks all the material on the 3' side of the 18S sequence. The cleavage that generates 32S and 20S precursors must take place within the central non-rRNA region, since 32S RNA has 5' terminal 28S sequence and 3' terminal non-rRNA sequence; and 20S RNA has a 5' non-

rRNA sequence with 18S rRNA located 3' terminally.

Each ribosome contains one 5S rRNA molecule in addition to the major RNAs and this seems to be coded by genes dispersed in the genome whose transcription is independent of that of the main precursor. The presence of another small RNA was first revealed by Pene, Knight and Darnell (*J. molec. Biol.*, **33**, 609–623; 1968), when they found that denaturing treatments release a small fragment from 28S rRNA. Originally characterised as 7S RNA, this molecule must be covalently linked to 28S rRNA, and more accurate characterisation has led to its recent description as 5.8S RNA. Synthesis of 5.8S RNA seems to be linked to that of the large rRNAs—kinetic experiments suggest its derivation by cleavage from the 32S precursor in HeLa cells. The sequence of the HeLa 5.8S RNA has now been examined by Maden and Robertson (*J. molec. Biol.*, **87**, 227–236; 1974), who have compared the oligonucleotides released by T1 ribonuclease with those cleaved from the rRNA precursors. When 28S rRNA is prepared by cold phenol extraction, it retains the 5.8S fragment; but heat treatment separates 28S rRNA from its small attached molecule. Correspondingly, upon T1 ribonuclease analysis, these two preparations of 28S RNA differ in the oligonucleotides characteristic of 5.8S RNA. One equivalent of the 5.8S RNA sequences is present for every equivalent of 28S RNA.

Fingerprints of the 32S RNA precursor show that it possesses the oligonucleotides constituting 5.8S RNA, again in molar yields which suggest the presence of one 5.8S RNA sequence in each precursor molecule. Some evidence for the presence of these fragments in 45S RNA is also presented. Although not yet precisely placed within the precursors, the 155 nucleotide long 5.8S RNA presumably lies between

Multifunctional gene in a eukaryote

ON page 630 of this issue of *Nature* Bollon presents a genetical analysis of a "multifunctional" eukaryotic gene, threonine deaminase, concerned with both structure and regulation.

Working with the yeast, *Saccharomyces cerevisiae*, Bollon found that the *ilv1* gene not only encodes for a gene product that is catalytically active (that is, L-threonine deaminase; EC4.2.1.16) but also that the gene product is involved in multivalent repression of the other isoleucine-valine enzymes. It has thus been possible, by careful genetical analysis, to discriminate between the nucleotide sequences that encode for that structural (=catalytic) and regulatory (=multivalent repression) functions of the *ilv1* gene. The advantage of using the yeast system, for studying regulatory phenomena, is that yeasts lend themselves well for intragenic complementation. Using many different *ilv1* mutants—impaired in threonine deaminase activity—intragenic complementation and fine structure analyses have been carried out.

Goldberger (*Science*, **183**, 810–816; 1974); Levinthal *et al.* (*Nature new Biol.*, **246**, 65–68; 1973) and Kasai (*Nature*, **249**, 523–527; 1974) have provided excellent background information about gene regulation. The first suggestion of an enzyme participating in its own regulation—by repression—was made by Vogel (in *The Chemical Basis of Heredity*, edit. by McElroy and Glass, Johns Hopkins Press, Baltimore 1957, page 276); it is also pertinent to note that the yeast system developed by Bollon and Magee (*Proc. natn. Acad. Sci. U.S.A.*, **68**, 2169–2173; 1971; *J. Bact.*, **113**, 1333–1344; 1973) offered the first evidence *in vivo* for the regulatory role of threonine deaminase, or for some form of the

ilv1 gene as Bollon shows in this issue. Of course, Umbarger and his many colleagues, as well as Hatfield's group, have provided a vivid picture of the role of *ilvA* (which specifies threonine deaminase) in multivalent repression in bacteria.

Although the *ilv1* gene product in yeast seems to be involved in multivalent repression, other elements are involved too. As already mentioned, Bollon and Magee noted that the involvement of leucine in multivalent repression may be unique (compare Levinthal *et al.*, 1973) and they suggest that leucine may function in repression by way of leucyl-tRNA; thus the *ilv1* gene product may be only one component of the multivalent machinery. One vital feature of the yeast work which may have been overlooked is that the genes specifying its various isoleucine-valine enzymes are found on different chromosomes (compare *ilvADE*, *ilvB* and *ilvC* in *Escherichia coli*).

The principal points of Bollon's work can be summarised as follows:

- A multifunctional gene (*ilv1*) has been analysed in detail in a eukaryotic organism for the first time.
- The *ilv1* product, threonine deaminase, catalyses the conversion of L-threonine → α -ketobutyrate in yeast.
- The regulatory role of the *ilv1* gene product is considered a positive effector for the derepression of the isoleucine-valine enzymes.
- Thus *ilv1* is truly 'multifunctional' with catalytic and regulatory parts being played by threonine deaminase.
- Intracistronic discrimination denoting the catalytic and regulatory functions of *ilv1* is presented.

GERARD A. O'DONOVAN

the 28S and the 18S sequences, and the 5' side of the point where cleavage takes place later to generate 32S RNA. The single methyl group present in 5.8S RNA is also present in 32S and 45S precursor RNAs; this is consistent with the idea that methylation is confined to rRNA sequences in the precursors. Furthermore, two other sites of modification contain pseudouridine; it is not yet confirmed that the modification is introduced into the precursor, although this seems likely. Modification at one of these sites may be incomplete. Future determination of whether the 5.8S sequence is contiguous with the 28S sequence in the precursor, or whether there is a space between them corresponding to a non-rRNA sequence, may help to decide upon models for processing and the possible implication of secondary structures or particular nucleotide sequences. Whether the 5.8S RNA sequence is noncovalently bonded to the 28S sequence in the precursor or takes up this association after maturation is another relevant question.

Another approach to defining the organisation and synthesis of RNA sequences is to describe their location in the genome. By hybridising *Xenopus* DNA with 5.8S RNA, Speirs and Birnsteil (*J. molec. Biol.*, **87**, 237-256; 1974) found that the relationship of 5.8S to 28S and 18S sequences seems to be similar to that in HeLa cells. When *Xenopus* DNA is fractionated, the rDNA containing the sequences coding for 28S and 18S rRNAs can be separated from the bulk of the genome by its distinct content of G-C base pairs, a technique used some time ago by Brown and Weber (*J. molec. Biol.*, **34**, 681-698; 1968 and Birnsteil *et al.* (*Nature*, **219**, 454-463; 1968) to show that 28S and 18S sequences alternate and each pair is separated from the next by a spacer. Speirs and Birnsteil find that the fraction of a density gradient hybridising with 5.8S forms a satellite with the density characteristic of rDNA. The conclusion that 5.8S coding sequences are interspersed with 28S and 18S sequences is suggested also by their observation that the nucleolate mutant of *Xenopus* (which lacks both the 28S and 18S genes) does not synthesise 5.8S RNA; nor does 5.8S RNA hybridise with DNA extracted from mutant cells.

Because DNA-RNA hybrids have a buoyant density different from that of denatured DNA, the extent of the density shift when DNA fragments are annealed with rRNA reveals the proportion of the fragment complementary to RNA. And in linkage experiments, the DNA hybridising with one rRNA can be tested for its ability to hybridise with the other. By using DNA fragments of different lengths, it is possible to follow the organisation

of sequences in DNA that code for these RNAs. Extending the previous experiments of this nature with 28S and 18S rRNA to 5.8S RNA, Speirs and Birnsteil observed a small density shift when DNA fragments of 8 and 3 times its length are hybridised with 5.8S RNA—the low density shift implies that 5.8S coding sequences are interspersed with other sequences rather than clustered together. Linkage experiments show that prehybridisation with 28S or 18S rRNA induces a large shift in the density of the fragments hybridising with 5.8S RNA; 18S rRNA causes a smaller shift than 28S rRNA, so that the 5.8S sequence must be closely linked to the 28S sequence and less closely related to the 18S sequence. Taken together with the analysis of HeLa rRNA precursors, these results suggest that the 5.8S sequence lies between the 28S and 18S sequences in the genome. The model which Speirs and Birnsteil present for the rRNA sequences in the genome postulates a small separation between the 3' end of the 28S sequence and the 5' end of the 5.8S sequence, with another separation between the 5.8S and 18S sequences.

The role of the 'transcribed spacer' regions which separate the sequences constituting the mature rRNAs is not clear; it is tempting to speculate, however, that it is concerned with folding of the rRNA regions or their interactions with proteins during maturation. Perhaps against this model is the observation that the mature RNA sequences are very similar in length in, for example, *Xenopus* and HeLa cell, but the nontranscribed spacer lengths are very different. And in *Escherichia coli*, although the bacterial precursors are only slightly larger than the mature RNA molecules, ribosome assembly seems to take place perfectly well with isolated 23S and 16S rRNAs. A second type of spacer, the 'nontranscribed spacer', separates the precursor-coding sequences in nucleolar DNA and its function also is obscure, although recent experiments encourage the speculation that these sequences may in some way be concerned with the maintenance of identity in all the repeated rRNA sequences in the genome.

BENJAMIN LEWIN

New 30S ribosomal assembly map

from a Correspondent

THE updated version of Nomura's *Escherichia coli* 30S ribosomal "assembly map" (Held, Ballou, Mizushima and Nomura, *J. biol. Chem.*, **249**, 3103; 1974) raises two interesting points. First, of course, the additions to the map are themselves important, particu-

larly the inclusion of protein S12, which was absent from the original map (Mizushima and Nomura, *Nature*, **226**, 1214; 1970) and which is now receiving more and more attention (see for review Pongs, Nierhaus, Erdmann and Wittmann, *FEBS Lett.*, **40** Suppl., 28; 1974). The role of S12 in the streptomycin effect has been widely investigated, and although mutations in S12 confer streptomycin resistance (see, for example, Ozaki, Mizushima and Nomura, *Nature*, **222**, 333; 1969), it seems that proteins S3 and S5 are the ones which actually bind the antibiotic (Schreiner and Nierhaus, *J. molec. Biol.*, **81**, 71; 1973). Furthermore, revertant mutants from streptomycin dependence to independence show alterations in proteins S4 and S5 (Hasenbank, Guthrie, Stöffler, Wittmann, Rosen and Apirion, *Molec. gen. Genet.*, **127**, 1; 1973). This suggests a strong relationship between proteins S5 and S12, and it is therefore gratifying to find an interaction between these two proteins in the new assembly map.

Second, there is the question of which proteins are RNA-binding. The evidence presented by Held *et al.* that S17 (which now incidentally has become a separate entity from S16, the two proteins being treated as one in the original assembly map) can specifically bind to 16S RNA under reconstitution conditions is convincing. But in view of the controversy (discussed in detail by Held *et al.*) which has occurred over this protein and also S13, it is perhaps worthwhile to consider precisely what the term 'RNA-binding protein' means. The term normally defines proteins which can bind singly to ribosomal RNA under reconstitution conditions. The reconstitution buffer is necessary to obtain a specific interaction, but it must be remembered that this is a high salt buffer which reduces electrostatic interactions. This has the effect not only of preventing non-specific interactions, but also of weakening the specific RNA-protein interaction. Held *et al.* say that "it is conceivable that proteins other than the initial binding proteins also interact with 16S RNA in the finished ribosome structure". Perhaps it would be more appropriate to say that (in a particle which is two-thirds RNA, and whose proteins are almost all basic) it is almost inconceivable that other proteins do not interact with the RNA in the final structure.

This has of course been suggested already (see, for example, Lutter, Bode, Kurland and Stöffler, *Molec. gen. Genet.*, **129**, 167; 1974), although few people would go as far as Cox and Bonanou (*Biochem. J.*, **114**, 769; 1969) in arranging all the proteins in neat rows with identical RNA environments. It seems likely that the conclusion will be that in physiological salt conditions

the proteins have a widely varied but positive interaction with the RNA, with no sharp distinction between "binding" and "non-binding" proteins; the very existence of the controversy over the specificity of S17 and S13 binding supports this view.

Organic pollutants in the sea

from a Correspondent

THE requirements of industry and of agricultural practice have in recent years greatly enlarged the search for and use of a variety of classes of organic compounds which in large quantities eventually reach the sea. The nature, origin, dispersal persistence and fate of some of the more commonly occurring pollutants including halogenated hydrocarbons, organic heavy metal compounds and crude oils were reviewed at a discussion meeting organised by the Royal Society on July 4-5.

N. S. Thom and A. R. Agg (Water Research Centre, Stevenage) cited more than 200 substances believed to be significant pollutants of water in the United Kingdom, and C. R. Pearson and G. D. McConnell (ICI Ltd, Brixham and Runcorn) reviewed a range of industrial hydrocarbons in widespread use. It seemed from the papers of E. D. Goldberg (Scripps Institution, La Jolla) and J. E. Portmann (Fisheries Laboratory, Burnham-on-Crouch) that although the input of both DDT and dieldrin into the oceans of the northern hemisphere may have passed its peak, the output is increasing in the equatorial regions and southern hemisphere and world production of organochlorine pesticides is increasing. Although, as Pearson and McConnell pointed out, the many kinds of chlorinated hydrocarbons derived from C_1 and C_2 hydrocarbons used as intermediates in further manufacture or as solvents and carriers are not unexpectedly dispersed to the sea substantially through the atmosphere, heavier synthetic halogenated hydrocarbons such as PCBs and DDT residues may similarly be transported as gas molecules or adsorbed on airborne dust. Goldberg and Portmann each suggested a 25% transference to the oceans by these means.

Within the sea persistence of organic pollutants varies greatly according to their chemical stability, susceptibility to biodegradation and the routes and terminal situations of their passage. Half lives varying from a few hours (for example, CCl_4) to days or weeks (EDC tars deriving from vinyl chloride production) to years (for example, PCBs, DDT, aromatic compounds of high carbon number) were cited by S. Jen-

sen (Wallenberg Laboratory, Stockholm), R. Lange (Odense University) and E. D. S. Corner (Marine Biological Association, Plymouth) among other speakers as examples of varying persistence.

G. Eglinton (University of Bristol) showed a film demonstrating a newly developed computerised gas chromatography-mass spectrometry system used for the determination and assay of pollutants in coastal muds, a system which may prove to be useful also in determining the natural occurrence of organic compounds in muds deposited in pre-industrial times.

The uptake of organic pollutants by marine organisms and their levels of occurrence and sites of accumulation were discussed by several speakers. Their differential absorption by lipids was stressed and Portmann made the point that although many measurements had been made of the concentrations of the more persistent compounds in a variety of organisms of the higher trophic levels, little was known of transfers at lower trophic levels, for example in the grazing of phytoplankton by zooplankton. Corner gave an account of work in progress on the utilisation, transformation and breakdown of fossil fuel hydrocarbons by marine crustaceans, a subject of particular interest in studies of the effects of oil spills. D. E. Hughes and P. McKenzie (University College, Cardiff) reported laboratory and field experiments indicating that about 40-90% of oil may be degraded by microbial attack; alkanes and other saturated compounds are more readily degraded than aromatic and heterocyclic compounds. A. J. Van Benekom (Netherlands Institute for Sea Research, Texel) reported on some consequences of discharges from the Rhine on the chemistry and plankton production of coastal water of the Southern Bight of the North Sea. Diatom growth was at times inhibited and the general level of production was unusually low and subject to irregular peaks. Blooms of *Phaeocystis* and possibly other toxic flagellates seemed to be favoured by these conditions.

New model of lunar interior

from Peter J. Smith

ACCORDING to Toksöz *et al.* (*Science*, **176**, 1012; 1972), the Moon has a 50-60 km thick crust in the eastern part of Oceanus Procellarum; and Nakamura *et al.* (*Science*, **181**, 49; 1973) concluded that the deep lunar interior below a depth of about 1,000 km is probably partially molten. But what is the state of the Moon between these

two extremes, in the mantle which accounts for the major part of the lunar volume? The complete answer to this question is not yet available, although Nakamura *et al.* (*Geophys. Res. Lett.*, **1**, 137; 1974) have now moved some way towards it in reporting new seismic data from the four Apollo seismic stations still in operation.

Nakamura and his colleagues have attempted to determine both P and S wave velocities throughout the lunar mantle from large meteoroid impacts, high frequency teleseismic events and deep moonquakes. The observed P wave velocity shows a systematic variation with epicentral distance, indicating that the subcrustal region cannot have a constant velocity. For example, P wave arrival times at distances greater than 70° are delayed by 10 s with respect to those expected from data at shorter ranges—an offset which could arise from a negative velocity gradient in the upper few hundred kilometres of the mantle, from a small stepwise decrease in velocity at a depth of the few hundred kilometres, or from a combination of the two.

For a negative velocity gradient, the travel-time curve would be continuous between 50° and 70°; and on this assumption, Nakamura *et al.* calculate that the P wave velocity would decrease from about 8.1 km s⁻¹ at the top of the mantle to about 7.8 km s⁻¹ at a depth of about 500 km, followed by a slight increase. For a stepwise decrease, the travel-time curve would be discontinuous with a shadow zone somewhere between 50° and 70°; and calculations put the minimum depth of the step at 200-300 km and its velocity contrast at 0.3 km s⁻¹. For a combination situation, the velocity gradient and velocity contrast would be smaller than those I have given. Unfortunately, the available data are too uncertain to enable a decision to be made about which of the three cases actually obtains, although Nakamura and his colleagues seem to favour the combination.

More certain (though based on a single data point) is a very large delay in P wave arrival at 168° which, if subsequently confirmed, would suggest that the Moon has a low velocity core. The present data put a limit of 170-360 km on the radius of such a core and a limit of 3.7-5.1 km s⁻¹ on its P wave velocity. Also conspicuous is the near linearity of the S wave travel-time curve beyond distances of 90°, indicating a large, and apparently increasing, delay of S wave arrivals relative to P wave arrivals at greater distances. Possible explanations involve one or more of the following effects: refraction of waves by a large negative velocity gradient at about 300 km depth, diffraction of waves around a discontinuity at this depth, or penetration of

waves into a lower velocity zone below. In fact, calculations suggest that the S wave velocity decreases from about 4.7 km s^{-1} at the top of the mantle to about 4.4 km s^{-1} at 300 km, followed by a more rapid decrease to a value of $3.8 \pm 0.2 \text{ km s}^{-1}$. Below 800 km, S waves are highly attenuated.

From these and other results, the most up-to-date picture of the lunar interior is then as follows:

- Zone 1: crust. This uppermost zone is 50–60 km thick in the region of the Apollo 12 and 14 stations and has seismic velocities consistent with plagioclase-rich material. The top few hundred metres is highly pulverised.
- Zone 2: upper mantle. This zone is about 250 km thick and has a P wave velocity of about 8.1 km s^{-1} which probably decreases slightly with depth. The seismic data are consistent with mineral assemblages comprising olivines (mostly) and pyroxenes (partly). The temperature gradient at the top of this zone is calculated to be $2\text{--}5^\circ \text{C km}^{-1}$.
- Zone 3: middle mantle. This is a zone with reduced S wave velocity and extends from 300 km to 800 km. Deep moonquakes occur at the base of it.
- Zone 4: lower mantle. This zone below 800 km is marked by high S wave attenuation and is possibly partially molten (as the terrestrial asthenosphere).
- Zone 5: core. This zone has a radius of 170–360 km and is marked by a greatly reduced P wave velocity. Molten?

Microclimate of open shade habitat

from Peter D. Moore

EXTREME habitats and the morphological and physiological adaptations necessary for life within them have always been more popular topics for study than have moderate conditions. It is becoming apparent, however, that immediate habitats, or ecotones, often have interesting attributes which make them worthy of special attention. An example of such a situation is provided by the study of open and shaded habitats.

For many years it has been conventional to classify plants into sun-demanding (heliophytes) and shade-requiring (sciophytes), and a great deal of research has been carried out both upon the physiological adaptation of these plants (for example, Björkman and Holmgren, *Physiologia Pl.*, **16**, 889; 1963; Björkman, *ibid.*, **19**, 854; 1966) and upon the light climates of shade habitats (for example, Anderson, in *Plant Photosynthetic Production*, edit. by Sestak, Catsky and Jarvis, Junk, The Hague, 1971). Little atten-

tion, however, has been given to intermediate situations, which can be termed 'open shade'.

A study of the open shade habitat from the microclimatic point of view has now been made by Stoutjesdijk (*Acta bot. neerl.*, **23**, 125; 1974). Surface temperatures on the north side of a patch of hawthorn (*Crataegus monogyna*) scrub in a Dutch sand-dune system were found to be as much as 10°C below those of ambient air. Although surfaces temperatures above ambient are commonplace, to find surfaces considerably below ambient at midday is extremely unusual and can only be explained in terms of the overall energy balance in these situations. At 11.30 h on a bright day in September, Stoutjesdijk found the surface temperature to be 9.1°C whereas ambient air was 15°C . Incoming radiant energy was in the form of diffuse solar radiation ($0.1 \text{ cal cm}^{-2} \text{ min}^{-1}$) and long wave radiation from the sky ($0.395 \text{ cal cm}^{-2} \text{ min}^{-1}$). Radiant energy loss was accounted for by reflection ($0.01 \text{ cal cm}^{-2} \text{ min}^{-1}$) and by long wave radiation ($0.523 \text{ cal cm}^{-2} \text{ min}^{-1}$). The net outcome of this situation is negative energy balance of $-0.038 \text{ cal cm}^{-2} \text{ min}^{-1}$, this being largely the result of the high long-wave radiation losses. The negative radiation budget is rather weak, however, and cannot fully account for the low surface temperature. The true energy budget is more strongly negative because of latent heat losses from the surface during the evaporation of dew; this explains the low surface temperatures.

This type of energy balance situation can occur only in open shade, for only here are long-wave losses large in comparison with gains and evaporation can take place at a rate sufficient to maintain high latent heat losses. Surface temperatures were found to be below ambient in daytime throughout the year, but differences were least in June and July (shading effects diminished) and greatest in late summer (strong shading and considerable dew formation).

Some work has been carried out on the influences of leaf canopies upon the spectral quality of light, but most of this has been concentrated on fairly dense canopies (see, for example, Federer and Tanner, *Ecology*, **47**, 555; 1966; Daynard, *Can. J. Bot.*, **47**, 1989; 1969; *Acta bot. neerl.*, **21**, 185; 1972). These studies have shown that the rate of visible to far-red radiation decreases considerably as shading increases. Measurements by Stoutjesdijk in his open shade habitat showed that there was a far better penetration of photosynthetically usable light ($<700 \text{ nm}$) than in the deep shade beneath hawthorn scrub.

Open shade habitats, then, provide

an interesting combination of physical conditions—low daytime temperatures (with consequent high-relative humidities) and moderate photosynthetically-effective light levels. Undoubtedly some plant species characteristic of open shade habitats, such as, for example, *Mnium undulatum*, *Rhytidiadelphus triquetrus*, find such conditions to their liking. Some anomalous plant distributions may also be explicable in terms of this unusual combination of microclimatic factors. For example, it may be that the steep, north facing scarp slopes of the South Downs provide a similar microclimatic habitat to Stoutjesdijk's 'open shade'. In which case it might explain the persistence in such situations of montane bryophyte species, such as *Racomitrium lanuginosum*, which are known to require high humidities, fairly low temperatures and moderately high light intensities for their survival.

Nature of Hoag's object

by John Gribbin

IT is now nearly a quarter of a century since Hoag discovered a remarkable astronomical object which appeared to be a "perfect halo" surrounding a diffuse nucleus. But only now is Hoag's object, as it has come to be known, receiving the attention which its peculiarity merits. According to O'Connell, Scargle and Sargent (*Astrophys. J.*, **191**, 61–62; 1974) the object is certainly worth investigating and "one of the more exotic possible interpretations" is that the ring is the image of a background galaxy produced by a gravitational lens effect.

Hoag's object is clearly visible on *Sky Survey* prints (at $\alpha = 15 \text{ h } 15.0 \text{ min}$; $\delta = +21^\circ 46'$) but it has not previously been studied in detail. The core of the object is a fuzzy circle $6''$ in diameter, and the surrounding blue ring or annulus has inner diameter $28''$ and outer diameter $45''$ with an axial ratio of 0.93 ± 0.05 . The ring is structureless, and there is no sign of connecting material bridging the gap between core and annulus.

In the study now reported by O'Connell *et al.* new information has been obtained from spectrograms taken with the 200-inch telescope and the Lick 120-inch. The observed redshift of $12,740 \text{ km s}^{-1}$ and an assumed Hubble constant of $75 \text{ km s}^{-1} \text{ Mpc}^{-1}$ imply a distance of 170 Mpc, corresponding to a linear diameter of 5 kpc for the core and inner and outer radii of 23 kpc and 37 kpc for the ring.

Almost all of the properties of the core seem to be in line with those expected for compact galaxies, but there seems little doubt that the ring is "an

independent structural component . . . and not simply the unobscured rim of a normal SO disk". Several other similar systems are known, and O'Connell *et al.* cite the examples of NGC6028, NGC2859 and IC5285. "There is no reason to suppose that radiation from these rings is anything other than direct starlight", and in the case of Hoag's object the radiation certainly cannot be reflected light from the core, since the ring is "at least" as luminous as the core.

The interpretation which seems to be favoured by O'Connell *et al.* involves gravitational encounters between galaxies which could initiate star formation in "trapped" rings; they point out that a pair of galaxies near Hoag's object on the sky could, if they are at the same distance as the object, have passed by it 10^5 to 10^8 yr ago, just right to account for the apparent age of the stars in the ring.

Merokeratins

from Peter Speakman

Wool is a mixture of about 100 different proteins, but Haylett's group in South Africa have been able to purify several proteins with a high cysteine content and to show homologies in their amino acid sequences. Argument is now over whether these proteins have evolved from a repeating penta- or deca-peptide (Swart and Parris, *Nature*, **249**, 580; 1974). Lindley and Cranston have pointed out that because of the heterogeneity of proteins a new experimental approach is needed to supplement sequence studies in order to study the pattern of disulphide crosslinks between proteins as they are arranged together in the fibre (*Biochem. J.*, **139**, 515; 1974).

Protein fragments which are very similar in molecular weight, dimensions and α -helix content have been prepared from mammalian epidermal keratin and from reduced wool by partial proteolysis, the method which was used to prepare the meromyosins and more recently, myosin subfragments 1 and 2. A sharp low-angle X-ray diffraction pattern from oriented films of the wool fragment showed that it could aggregate in a specific way into long, thin assemblies. These fragments from epidermis and reduced wool may be in the native conformation; and so studies of their internal structure and dimensions in solution and investigations of their specific aggregates, are beginning to give information about the conformation of the protein chains within the keratin macromolecular components, and about the mutual positions of the macromolecules in the fibre or tissue. Analogous studies of dissolved muscle proteins and their modes of aggrega-

tion, and of tropocollagen and its polymorphic aggregates have contributed to a very detailed understanding of muscle and connective tissue structure.

The small-angle X-ray diffraction pattern published from the CSIRO Division of Protein Chemistry in Melbourne shows that a wool keratin fragment can be made to form specific aggregates which are about 200 Å in diameter and greater than 2,000 Å long, with a longitudinal repeat of 160 Å (Suzuki *et al.*, *J. molec. Biol.*, **73**, 275; 1973). The method of preparing the fragment is interesting and relevant. Wool proteins are dissolved by reduction in a solution of a denaturing agent (8M urea). The thiol groups are blocked by reaction with iodoacetate ions, and the urea is removed by dialysis. The proteins (SCMKA) with a smaller proportion of cysteine residues and a higher α -helix content than the original wool are separated by fractional precipitation and purified by chromatography.

It is clear that some refolding of the SCMKA proteins, into conformations which are not completely flexible, takes place when the urea is removed, because a fairly large protein fragment (as well as small peptides) is produced when the SCMKA proteins are treated briefly with chymotrypsin. The fragment has a molecular weight of 41,000, a length of 170–200 Å measured in solution or 160 Å in the electron microscope, and a diameter of 20 Å. It has a higher α -helix content (80%) than the original SCMKA proteins (50%) and it is believed to consist of three chains (Crewther and Harrap, *J. biol. Chem.*, **242**, 4310; 1967; Dobb *et al.*, *J. Textile Inst.*, **64**, 374; 1973). A similar wool fragment has been prepared without using a denaturing agent (Hilburn *et al.*, *Biochim. biophys. Acta*, **214**, 245; 1970; Dilley *et al.*, Abstracts, British Biophysical Society Meeting, Leeds, April 1971). If wool is reduced at pH 10 (which itself may cause some denaturation) and treated with trypsin, a rod-shaped fragment, 200 Å long and 20 Å wide, molecular weight 55,000, with a higher α -helix content (64%) and a lower cysteine content than the original wool, can be separated from the digest.

An analogous fragment prepared by partial proteolysis of epidermal keratin protein is reported from Matoltsy's laboratory (Skerrow *et al.*, *J. biol. Chem.*, **248**, 4820; 1973). It is not necessary to reduce disulphide crosslinks in order to extract protein from mammalian epidermis but a high or low pH is needed (in this case pH 2.6), which may again cause some denaturation. The solution of epidermal protein in acid buffer is added dropwise to a trypsin solution maintained at pH 8.8, and a protein fragment can be separated from the digest, length 200 Å, molecular weight 46,000, which is 83% α -helical.

Polyacrylamide gel electrophoresis in denaturing conditions showed that the fragment consisted of three chains with approximately similar molecular weights.

The protein chains of SCMKA were denatured during the preparation, and therefore information about the structure of wool can only be inferred from the structure of the SCMKA proteolytic fragment, and from the mutual arrangement of the fragments in the specific aggregate, if partial or exact renaturation occurred when the denaturant was removed. Similarly, the other wool fragment and the epidermal protein fragment have been exposed to pH values which might cause denaturation. On the other hand, the similarity between the three fragments strongly suggests that undenatured or accurately renatured parts of the keratin structure have been prepared in these experiments. Their compositions and α -helix contents imply that they originate in the wool protofibril and the epidermal protofilament, but the low yield in each preparation (for example, less than 10% in the case of the wool fragment prepared without a denaturing agent) means that they may not represent the whole of these structures.

This work will encourage attempts to dissolve native proteins from epidermis or reduced wool without the use of proteolytic enzymes or denaturing agents, and the study of their structure in solution. Fairly extreme conditions of pH or temperature dissolve large quantities of keratin proteins but they may cause denaturation. Milder conditions are less likely to cause denaturation, but smaller amounts of protein dissolve and it may not be clear whether they are minor components or partly soluble major components. In both cases, even if the protein has a definite conformation in solution this will not necessarily be identical to the conformation adopted by the same protein in the fibre or tissue. A similar problem arose in collagen research in the 1950s. Acid or neutral buffer solutions will only dissolve small amounts of tropocollagen from calf skin, for example, but it was possible to show that the conformation of the protein chains in dissolved tropocollagen is the same as in intact connective tissue. The length of the tropocollagen molecule measured in solution was shown to be consistent with the dimensions of one of its specific aggregates (SLS) in the electron microscope; and the mutual arrangement of the tropocollagen molecules in another specific aggregate (quarter-stagger) was deciphered, and shown to be very similar to the arrangement in connective tissue. Experiments with the native fibrous protein in solution, and research with intact tissue dramatically confirmed each other.

3C236, DA240; the largest radio sources known

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A high resolution study has been made of faint and very extensive radio components associated with the galaxies 3C236 and DA240. Their respective linear dimensions of 5.7 Mpc and 2 Mpc are without precedent. Their properties are compared with those of other radio galaxies and the consequences for present models of the evolution of these objects are briefly discussed.

It is important for several reasons to determine the maximum size to which a radio source may evolve. First, the relativistic electrons in the radio components will suffer unavoidable energy losses by various processes as they move away from the associated optical object^{1,2}. These losses are likely to be severe for very old or large sources and it may be that the study of such objects will allow a choice to be made between the various modes of energy replenishment that have been suggested³. Second, these sources might serve as probes of the intergalactic medium, for their structure is likely to be strongly influenced by it. Finally, radio components of large angular size can be mapped in detail to obtain much needed clues as to the physical processes occurring in them.

So we felt it essential to ascertain whether the apparent upper cutoff in radio source sizes at about 1 Mpc (refs 3, 4) is a real effect or largely a consequence of observational selection. We have begun a programme of observation at a wavelength of 49 cm with the Westerbork Synthesis Radio Telescope (WSRT) to search for very large sources

which had not been recognised as such in earlier observations. Such a search requires a telescope with good sensitivity, as well as a resolving power sufficient to both delineate the extent of large objects and reliably separate them from unrelated confusing sources. A telescope is most sensitive to extended emission at long wavelengths because of its greater beam area and the non-thermal spectra of the radio sources. The effective sensitivity of existing single dishes can be increased only marginally by observing at longer wavelengths because of the severe limitations imposed by confusion.

With an aperture synthesis instrument like the WSRT, however, we can take full advantage of the improvement expected from a lowering of the frequency. A change in observing wavelength from 21 cm to 49 cm renders the instrument ten times more sensitive to typical radio emission distributed over several synthesised beams. Confusion remains tolerable, with an average of one detectable source in about 200 beam areas, while the twenty baselines simultaneously obtained ensure a low level of 'grating response confusion'.

Source selection and first results

We have selected for study a number of confused regions listed in the catalogue of Bridle *et al.*⁵. Observing with 10' resolution at 21 cm, they were unable to decide whether the objects were composed of physically associated components, or unrelated sources. Here we report our first results; the sources 3C236 (1003+35) and DA240 (0744+55) are huge radio galaxies having overall sizes

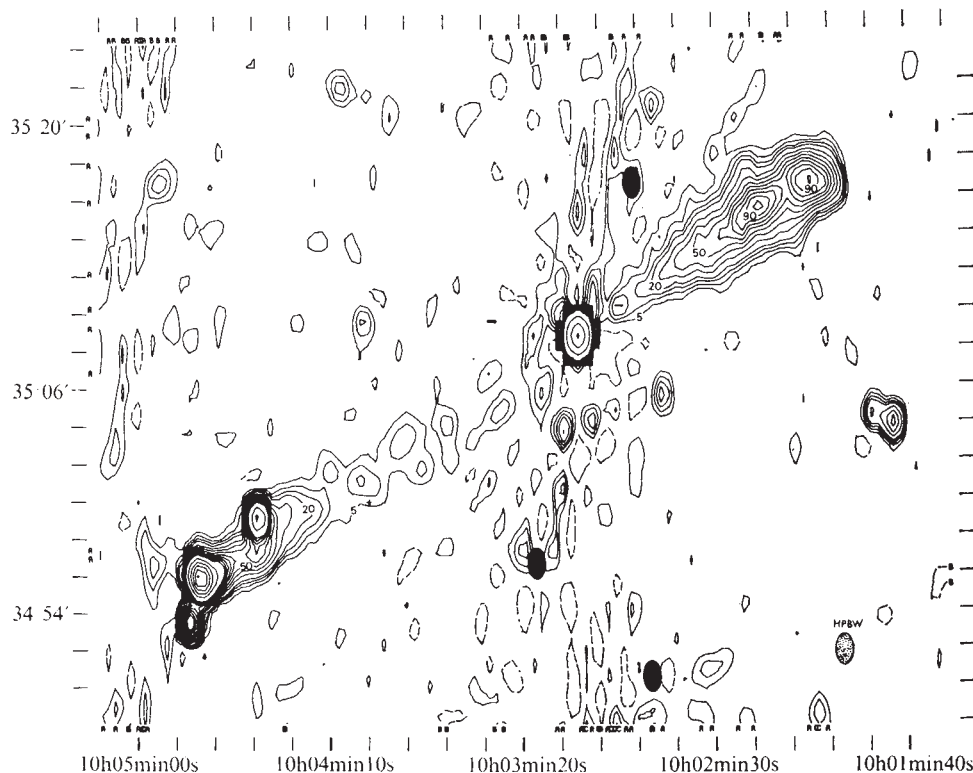


Fig. 1 Contour map of 3C236 at 49 cm. The field centre, at RA 10 h 04 min, Dec. 35° 00', is marked by a cross. The dashed contour is at a level of -7.5 mJy and continuous contours are plotted at intervals of 5 mJy per synthesised beam area from 5 mJy to 20 mJy, 10 mJy from 20 mJy to 100 mJy, and 50 mJy from 100 mJy to 350 mJy. Continuous contours at the 500, 1,500, 3,000 and 4,500 mJy level are also plotted. Stripes extending approximately north-south from the central intense source are due to instrumental effects as discussed in the text. The three black ellipses represent intense background sources, the effects of which have been removed from the map.

Table 1 Observations of 3C236 and DA240

	3C236	DA240
Observation length (half days)	1	2
No. of spacings, increment (m)	20, 72	40, 36
Synthesised half-power beamwidth ($\alpha \times \delta$)	57'' $\times$ 99''	57'' $\times$ 69''
Grating ring radius ($\alpha \times \delta$)	24' $\times$ 41'	47' $\times$ 57'
R.m.s. noise (mJy)	~ 1.5	~ 1.0
Primary beam pattern half-power width	82'	

of 5.7 and 2.0 Mpc respectively (assuming Hubble's constant $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and an Einstein-de Sitter cosmology).

Early interferometric observations⁶ indicated that most of the emission from 3C236 was contained within a very compact source $< 3''$ in extent. Wilkinson⁷ resolved this source with a baseline of $112,300 \lambda$ at 1,423 MHz and found that two unresolved components separated by $1''$ in position angle 119° gave the best model fit to his data. Wyndham⁸ identified the source with a 16 mag elliptical galaxy. Examination of the Palomar Sky Survey (PSS) prints yields no evidence that the galaxy is in a cluster. Only a few 19 mag objects are visible nearby, and another 16 mag elliptical is present some $4'$ to the north-east. Sandage⁹ measured a redshift $z = 0.0988$ for 3C236 based on a single emission line identified with $[\text{O II}] \lambda 3727$. His accurate photometric data¹⁰ showed that 3C236 lies very close to the line of best fit in the Hubble diagram for radio galaxies. Bridle *et al.*⁵ found two radio components separated by $34'$ straddling the compact source. They pointed out that if all the radio emission were associated, the overall size could be as great as 8 Mpc.

The source DA240 was resolved by Bridle *et al.* into a double whose components were extended and separated by $17'$. Caswell and Wills¹¹ identified 4C56.16, the stronger eastern component, with a 15 mag galaxy, number 9-13-66 in the catalogue of Vorontsov-Velyaminov and Arhipova¹² (hereafter referred to as V-V). This galaxy shows weak emission lines and has a redshift $z = 0.0356$ (ref. 13). Our observations show that instead the galaxy to be identified with DA240 lies midway between the two components found by Bridle *et al.*

Observations and reduction

3C236 and DA240 were observed at 49 cm with the WSRT, a detailed description of which is given elsewhere¹⁴. It consists of 12 equatorially mounted 25-m parabolic dishes situated along an east-west line. Telescope parameters and information relevant to the present observations are summarised in Table 1. Phase stability of the 49-cm system during a twelve hour observing period is about $\pm 1^\circ$, and the gain stability is certainly better than $\pm 1\%$. In our experience, the detection of weak emission having a brightness 0.003 of the most intense peak on the map is feasible with the present system; in the 3C236 observation, which is particularly well calibrated, this value has been extended to almost 0.001.

Two major problems had to be overcome in analyzing the data. First, it was found that both sources contain extremely intense components more than one hundred times as bright as the surrounding emission. Since the inner sidelobes of the synthesised antenna pattern are about 4% of the main response, sidelobes from the intense structure initially masked the low-level radiation. Second, in the case of 3C236, grating rings from some parts of the source distorted the emission from other parts. We have therefore used the CLEAN technique¹⁵, in which structure in the map is iteratively decomposed into a set of point sources, the effects of which are then removed from the map using the antenna pattern with its sidelobes and grating rings. Subsequent convolution of the point sources with a 'clean' antenna pattern leads to a map free from the effects of the subsidiary responses.

The reception pattern of the individual paraboloids causes emission from regions far from the field centre to be attenuated. The maximum effect occurs at the outer edge of the western component of 3C236, whose brightness is reduced by 30%. Flux densities and other tabulated data have been corrected for this effect, but the contour maps have not.

Maps and basic data

A contour map of 3C236 is shown in Fig. 1. The source consists of two extended components on opposite sides of the intense compact source, with outer edges at distances

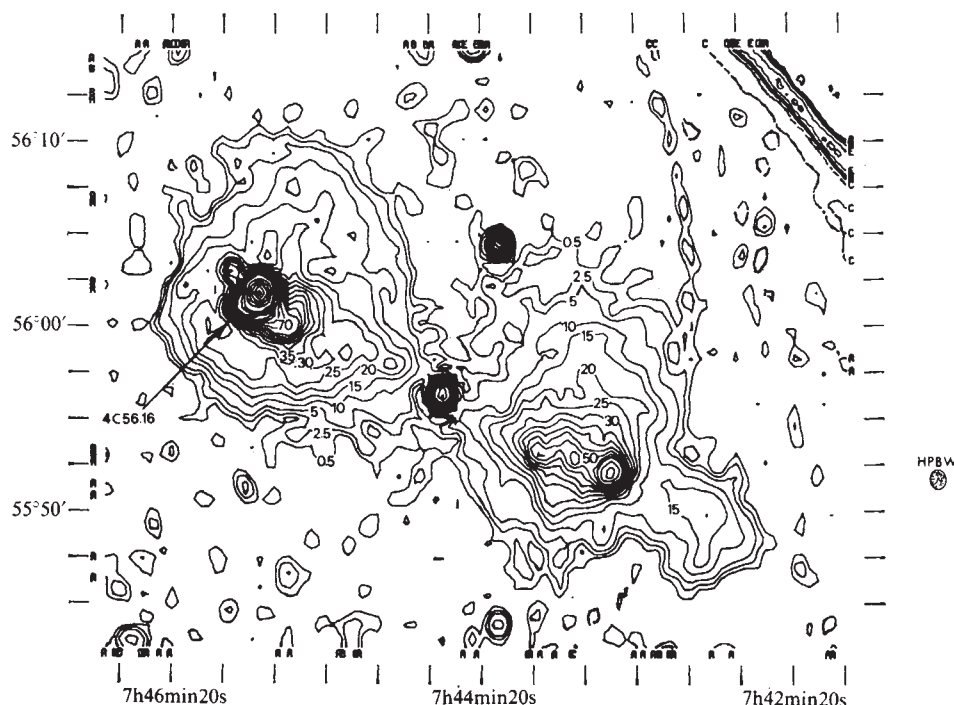


Fig. 2 A contour map of DA240 at 49 cm. The field centre, at RA 07 h 44 min 30 s, Dec. $55^\circ 55'$, is marked by a cross. The dashed contour is plotted at a level of -5.5 mJy and continuous contours are at levels of 0.5 mJy and 2.5 mJy per synthesised beam area, and then in steps of 5 mJy from 5 mJy to 60 mJy , 10 mJy from 60 mJy to 100 mJy , 25 mJy from 100 to 150 mJy , 50 mJy from 150 to 250 mJy and 250 mJy from 250 mJy to $1,500 \text{ mJy}$. The greatest contour is at a level of $1,600 \text{ mJy}$.

Table 2 Some basic data on 3C236 and DA240

	3C236	DA240
Position of central source (1950.0)	10 h 03 min 05.5 s $\pm$ 0.05 s 35° 08' 49.0" $\pm$ 0.6"	07 h 44 min 34.82 s $\pm$ 0.07 s 55° 56' 28.3" $\pm$ 0.6"
Redshift	0.0988	0.0356
Visual magnitude (uncorrected for absorption)	15.97 (ref. 10)	$\sim$ 15.2 (ref. 16)
Angular size along major axis (minutes of arc)	39	34
Size along major axis* (Mpc)	5.7	2.0
Size transverse to major axis (Mpc)	$\sim$ 0.5	$\sim$ 0.7
Size of central source (kpc)	2.44	$<$ 2.9
Flux density (mJy) (49 cm)	Central source 5.08 $\pm$ 0.05 East component 1.8 $\pm$ 0.2 West component 2.1 $\pm$ 0.2 Total 9.0 $\pm$ 0.3	0.267 $\pm$ 0.007 5.4 $\pm$ 0.3 3.3 $\pm$ 0.3 9.0 $\pm$ 0.5
Mean surface brightness of the outer components (mJy per synthesised beam) (49 cm)	37.4 $\pm$ 3.3	20.8 $\pm$ 1.2
Spectral index α ‡Integrated	-0.6 (ref. 18)	-0.77 (ref. 18)
Central source	-0.62 ± 0.03 (ref. 17)	-0.40 ± 0.06
49-cm integrated polarisation (%)	< 1.3	8.3 $\pm$ 1.3
at position angle (°)	—	118 $\pm$ 2

*Assuming $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and an Einstein-de Sitter cosmology.

†The flux densities are derived assuming 3C147 has a 49-cm flux density of 37.78 Jy, from extrapolation of the data of Kellermann *et al.*¹⁷.

‡ α defined in the sense flux density $\propto$ frequency α .

of 24' and 15' from it. The extension of the outer components along the line joining them and the precision of the collimation (lines from the two prominent outer peaks meet the central one at an angle of 179.5°) indicate the association suggested by Bridle *et al.*⁵ to be real. Basic data relevant to 3C236 (and DA240) are summarised in Table 2.

The contour map shows a number of spurious, predominantly radial features emanating from the central component, the most prominent of which are nearly vertical. These stripes are instrumental effects produced in the vicinity of intense point sources by small gain and phase fluctuations during the observation, and determine the dynamic range discussed earlier. The peaks beyond the south-east edge of the following component result from the same effect. Their narrowness and radial elongation are usually sufficient to distinguish such features from real structure.

The south-following component has three strong peaks near its south-east end. The brightest of these, with a maximum intensity of 364 mJy per beam area, is centrally located at the eastern end of the low brightness plateau. This component is resolved, except on its outer edge. We think it likely that the two other peaks are unrelated background sources for the following reasons. Both are unresolved and the southernmost one lies adjacent to, rather than within, the low brightness emission. Our polarisation data show the following component, including the main peak, to have significant linear polarisation; the two unresolved sources, however, are unpolarised. From the number of background sources stronger than 6 mJy in a typical WSRT field at 49 cm, we expect 3C236 to coincide, on average, with about 0.5 unrelated objects, which is not significantly different from the two suggested here. For the remainder of our discussion, the main peak will be assumed to define the eastern end of 3C236.

We find the position angle of the source axis to be 122.5°, very close to the 119° which Wilkinson* found for his model of the intense central double source. Since Wilkinson's value has an error of about $\pm 2^\circ$, the alignment may well be exact. This alignment shows that the process responsible for the double structure, which spans a linear scale ratio $\sim 2,000$, has maintained its orientation for at least 10^7 yr. It is also interesting that the position angle of the minor axis of the optical galaxy seems roughly coincident with the radio source axis.

The distribution of brightness in DA240 is shown as a contour map (Fig. 2) and as an intensity modulated radio photograph (Fig. 3), the latter being particularly suitable for the display of faint, extended regions. Like 3C236,

DA240 is dominated by a very intense source (4C56.16), so the map is slightly affected by limitations of dynamic range. Thus several faint stripes appear to radiate from 4C56.16, and there is a weak residual grating ring near RA07 h 43 min.

DA240 consists of two nearly circular diffuse components, each about 12' in diameter. These regions are more sharply bounded in the south than the north, suggestive of a gradient in the external gas density across the source. Each component has a dominant maximum, and these peaks are almost equidistant from an unresolved central component. These three peaks have widely differing intensities, the easternmost one (4C56.16) having a maximum surface brightness of 1.74 Jy per beam area. From its visibility curve at 49 cm, we find that 4C56.16 emits 1.4 Jy from a region smaller than 15". The westernmost peak is twenty times less intense than the 4C source and is fully resolved, even along its leading edge. Preceding the entire western component and clearly associated with it, is a faint elongated feature some 6' in length and 4' wide. The centre component has a flux density of 267 mJy at 49 cm and, according to our preliminary data, at 6 cm has an angular size less than 3" and a flux density of 115 ± 10 mJy.

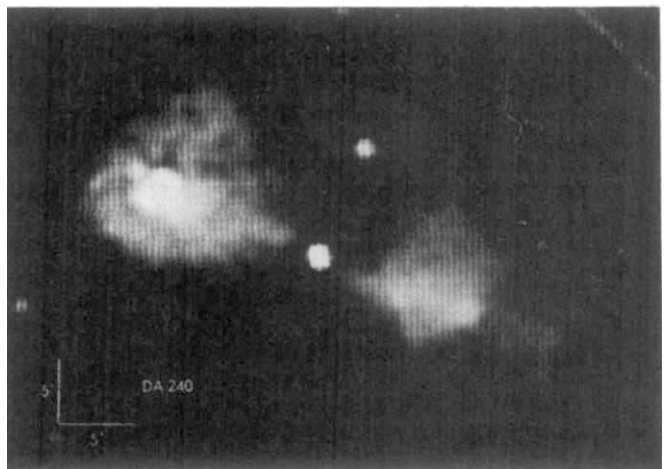


Fig. 3 An intensity modulated radiophotograph of DA240, produced on a rotating drum photo plot system designed by W. J. Jaffe. The declination scale is compressed by about 12% relative to the right ascension scale.

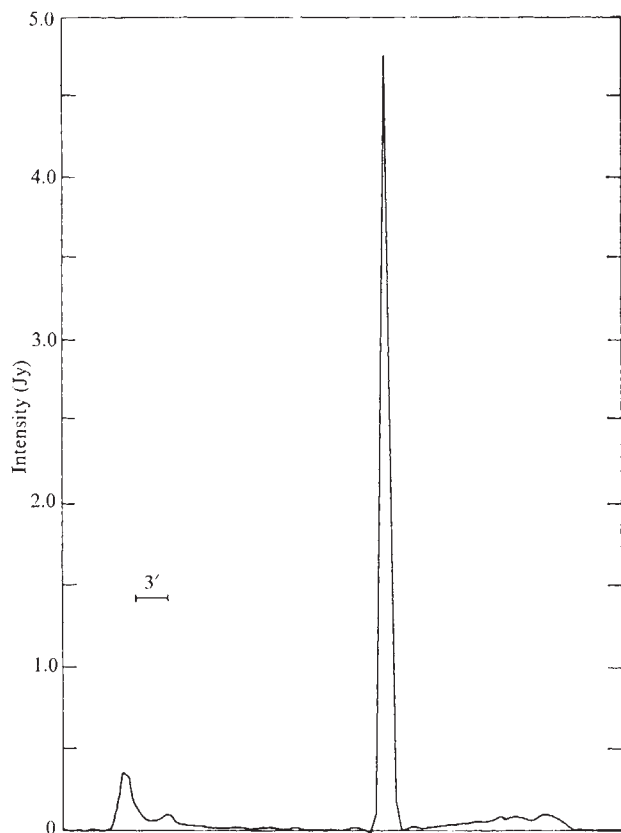


Fig. 4 A crosscut in position angle 122.5° along the major axis of 3C236.

We have identified the compact central source with an elliptical galaxy having a diffuse outer envelope (number 9-13-57 in the V-V catalogue). V-V gives the dimensions of the inner core as $30'' \times 24''$. Schmidt (private communication) has measured a redshift $z=0.0356$ for this galaxy.

On the north-east side of the intense component 4C56.16 there is an unresolved subpeak with an intensity of about 38 mJy which is coincident with the galaxy V-V 9-13-66, the object thought by Caswell and Wills¹¹ to be associated with 4C56.16 itself. The redshift ($z=0.0356$) given by Burbidge¹³ is exactly the same as that measured by Schmidt for V-V 9-13-57. V-V 9-13-66 is classified as "a flat galaxy whose arms have an indefinite direction of winding". The flux density implies a radio power of $1.5 \times 10^{22} \text{ W Hz}^{-1} \text{ sr}^{-1}$ at 49 cm, much stronger than that of normal spirals¹⁹. The tantalising question of whether V-V 9-13-66 is actually immersed in the eastern component of DA240 cannot be conclusively answered with the present data.

A ridge of emission extends from each outer component of DA240 towards the central one. The position angle of a line joining the end of each ridge near the central component is almost the same as the position angle of the major axis of the optical galaxy, giving the ridge system the shape of an open 'S'.

From the available data^{5,18} we have derived an integrated 178 MHz flux density of 22 Jy for DA240 which is well above the nominal completeness limit of the 3C survey. As shown by Bennett²⁰, however, the 3C observations are increasingly insensitive to sources whose angular size exceeds about $5'$. It is essential for workers deriving statistical properties of radio source samples to appreciate the inherent limitations of many source surveys in this respect.

Our observations also show that both 3C236 and DA240 have strong linear polarisation at 49 cm. Although the integrated polarisation of 3C236 is negligible, individual regions show degrees of polarisation up to 20%. The eastern peak is polarised by 10% but as we have already noted, the other two intense sources near it are unpolarised. Polarisations of up to 40% and possibly higher occur in the extensive low brightness regions of DA240. The intense unresolved structure in 4C56.16 is 22% polarised, and in its immediate surroundings similar values are found. DA240 has an integrated percentage polarisation at 49 cm of 8.3%, higher than previously found for any radio galaxy at this wavelength²¹. The polarisation of the central components, coincident with the optical galaxies are $< 0.4\%$ and $< 2\%$ for 3C236 and DA240 respectively.

Discussion

A remarkable feature of the 3C236 and DA240 maps, and the main reason that much of the structure has previously escaped detection, is the huge range of surface brightness. In 3C236 a ratio of more than 1,000:1 is found between the intense central source and the faintest emission in the south following component. A profile of intensity along the major axis of the source (Fig. 4) indicates these differences. Although DA240 does not display such an extreme disparity the peak intensity of 4C56.16 exceeds the average brightness of DA240 by more than a factor of 80. It is not yet possible to say whether these large variations in surface brightness, and the prominence of the central component at 49 cm, qualify 3C236 and DA240 as rare source types.

The ratio of the flux densities of the outer components and their relative distances from the optical galaxy are not unusual²² in these sources. It is, however, interesting to note that both outer components of 3C236 are well resolved transverse to the source axis, unlike the majority of 3C double sources when observed with a similar resolution²².

Table 3 Comparison of 3C 236, DA240, Cen A and Cyg A

Source	3C236	DA240	Cen A	Cyg A
Distance* (Mpc)	554	206	10	320
Linear size (Mpc)	5.70	1.99	1.57	0.16
$P_{\lambda_{49}}^{\dagger}$ ($\text{W Hz}^{-1} \text{ sr}^{-1}$)	2.6×10^{25}	3.6×10^{24}	2.1×10^{24}	2.7×10^{27}
Radio luminosity ($\sim$)	2.4×10^{36}	3.4×10^{35}	1.9×10^{35}	3.0×10^{38}
Component properties	east	west	east	west
Volume (10^{72} cm^3)	35	43	8.0	7.9
Minimum Energy [†] (10^{58} erg)	54	63	29	22
Energy density ($10^{-14} \text{ erg cm}^{-3}$)	1.6	1.5	3.6	2.8
Equipartition magnetic field [†] (10^{-7} G)	6.3	6.1	9.5	8.4
	north	south	east	west
Volume (10^{72} cm^3)	2.9	2.9	2.4×10^{-5}	1.9×10^{-5}
Minimum Energy [†] (10^{58} erg)	9.1	9.3	3.0	3.0
Energy density ($10^{-14} \text{ erg cm}^{-3}$)	3.1	3.2	1.3×10^5	1.6×10^5
Equipartition magnetic field [†] (10^{-7} G)	8.8	9.0	1800	2000

*Assuming $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and an Einstein-de Sitter cosmology.

[†]Minimum energy estimates have been made assuming the energy of cosmic ray protons to be negligible. If the energy in protons exceeds that in electrons by a factor of 100, the minimum energies and the equipartition magnetic field strengths must be multiplied by 13.9 and 3.7, respectively.

Table 4 Minimum energies ($U_{\min}$), equipartition magnetic fields (B_{eq}), thermal electron densities (n_e) and masses (M) of selected regions

Source	Region	$U_{\min}^*$ (erg)	B_{eq}^* (gauss)	n_e (cm^{-3})	M ($M_{\odot}$)
DA240	Intense eastern compact component	1.3×10^{58}	2.4×10^{-6}	$< 6 \times 10^{-5}$	$< 8 \times 10^8$
DA240	Extended eastern low brightness area	1.6×10^{59}	5×10^{-7}	$< 2.4 \times 10^{-5}$	$< 1 \times 10^{11}$
3C236	Eastern head	3.3×10^{58}	2.2×10^{-6}	$< 7 \times 10^{-5}$	$< 1.7 \times 10^9$

*Minimum energy estimates have been made assuming the energy of cosmic-ray protons to be negligible. If the energy in protons exceeds that in electrons by a factor of 100, the minimum energies and the equipartition magnetic field strengths must be multiplied by 13.9 and 3.7, respectively.

DA240, also, has unusual features. In many respects—the relaxed nature of the large outer components, the ‘S’-shaped ridge linking the brighter peaks—the source is reminiscent of Centaurus A (ref. 23). It may be significant that Centaurus A is also a large source exceeding 1 Mpc in length. Like 3C236, Centaurus A has a compact double source well within the large outer components.

In Table 3 we present a number of derived properties for 3C236, DA240, Cen A and the powerful radio galaxy Cyg A (ref. 24). We have assumed that the components are prolate spheroids whose depths are equal to their width in the plane of the sky along the minor axis and that the energy in cosmic ray protons is negligible. Examination of Table 3 shows that DA240 and Centaurus A do, indeed, have many similar properties. Cyg A, a much smaller, very intense source, has a higher luminosity, energy density and magnetic field strength than the other three.

In columns 1 and 2 of Table 4 we show the minimum energies $U_{\min}$ in the form of relativistic electrons and magnetic field, and the corresponding equipartition magnetic fields B_{eq} for two selected regions of DA240 and one of 3C236.

As we have already noted, both sources are strongly polarised at 49 cm. Stringent upper limits may therefore be placed on the thermal gas density in the sources, for high gas densities would cause depolarisation by differential Faraday rotation. Assuming the line of sight depths through the components to be equal to their size in the plane of the sky, and the magnetic field to take its equipartition value, we find the upper limits to the thermal density, n_e , given in column 3, while the corresponding masses are presented in column 4. The gas densities found are at most an order of magnitude greater than the cosmological density of 3×10^{-6} hydrogen atoms cm^{-3} , and could be very much less. Such low densities, and the large overall sizes of the sources have interesting implications for certain models of the evolution of extragalactic radio sources which we now discuss in turn.

De Young and Axford²⁵ proposed a dynamical containment mechanism in which the ram pressure of the intergalactic gas confines the radio emitting components. In the context of their model it has been suggested²⁶ that the extensive ‘tails’ of low brightness often seen to extend back towards the parent galaxy result from electrons which have diffused out of the compact outer components. Such a scheme does not seem applicable to DA240, for here the extended low brightness emission completely envelopes the compact regions. As the motion of the component is envisaged to be supersonic it is not clear how electrons could diffuse ahead of it. Of course, if the components were more or less stationary (and contained by some process other than ram pressure), the observed morphology could result. The ram pressure model may also be criticised on the grounds that it supposes the components to leave the galaxy with sizes not much smaller than their present ones. For 3C236, there is evidence from the double structure of the compact central source that the original component sizes are not bigger than 1 kpc. In the subsequent expansion to the observed diameter of ~ 150 kpc for the compact eastern component, adiabatic losses would decrease the flux by a factor of more than 10^3 . It is, therefore, necessary that energy in the form of magnetic field and relativistic particles

be distributed over volumes much larger than a galactic nuclear region in a loss-less way, probably to be supplied to the components throughout most of their lifetimes.

A useable feature of the model is that the components are continually decelerated and eventually brought to a halt after which the source expands rapidly. Christiansen²⁷ has shown that a plasmon can travel a distance

$$D = M / (\pi \times 1.67 \times 10^{-24} n_s r^2) \quad (1)$$

before dispersing. Here M is the mass of the plasmon, r its radius and n_s the number density of the gas surrounding the component. Although equation 1 was derived assuming ram pressure containment, it yields an estimate of the distance travelled by the plasmon before it is strongly decelerated even if it is contained by other processes. This is because the plasmon must, in any case, experience a retarding force of (ram pressure) $\times$ (cross-sectional area); r should then be interpreted as the component radius, suitably averaged over the source lifetime. In spite of uncertainty in this radius, it is interesting to apply equation 1 to the eastern component of 3C236 and so derive an upper limit to n_s by taking D and r as their present observed values and the upper limit to M from Table 4. We find $n_s < 10^{-6} \text{ cm}^{-3}$. If the source axis is not in the plane of the sky, the limit becomes correspondingly lower. In spite of this low density, the fact that the component leading edge is sharper than the trailing one suggests an interaction with an external gas. If the eastern component in 3C236 is, in fact, confined by ram pressure, $n_s > 3.6 \times 10^{-8} \text{ cm}^{-3}$, assuming the component velocity $v < 0.08c$ (ref. 28).

Gull and Northover²⁹ have proposed an elegant mechanism for the propulsion and confinement of radio components by a circumgalactic medium. The radially decreasing density of the medium, maintained by the galactic gravitational field, causes the components to ‘float’ away from the centre. The model seems, however, to be inapplicable to 3C236 and DA240 for several reasons. The most telling of these are the long component travel times ($\sim 10^{10}$ yr) and the difficulty of maintaining sufficient density gradients over the distances involved. Similar difficulties have been noted for Cyg A (ref. 24); for these giant sources they are even more severe.

Further observations of 3C236 and DA240 with the WSRT at 6 cm and 21 cm have either been planned or are already in progress. A presentation of these results and a full discussion of their implications will follow. We have also begun observations of other candidate objects, for it seems most improbable that these two are the only radio sources of moderate strength whose large scale structure has been overlooked. Before long we may know if 3C236 is atypical, or whether it must relinquish its distinction of being the largest known object.

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Fine structure analysis of a eukaryotic multifunctional gene

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The *ilv 1* gene of *Saccharomyces cerevisiae* contains functions associated with both structural and regulatory genes and therefore is termed a multifunctional gene. The *ilv 1* gene product catalyses the first step in isoleucine biosynthesis and is involved in repression of the isoleucine-valine pathway. The intracistronic discrimination of the two functions is presented.

In spite of considerable progress in the understanding of the underlying molecular mechanisms involved in the regulation of gene expression in prokaryotes¹, such mechanisms remain obscure in eukaryotes. Although several approaches for studying eukaryotic control mechanisms have involved animal systems², much information about eukaryotic regulatory phenomena has been obtained from studies with fungi. Work with *Neurospora* has revealed several complex forms of regulation^{3,4} and compartmentation⁵, and *Saccharomyces* has been the subject of several elegant studies⁶⁻⁹. Nevertheless, a regulatory macromolecule like a repressor protein involved in the control of gene expression in eukaryotic organisms remains to be identified and characterised.

There is evidence from *in vivo* studies that threonine deaminase or some form of the *ilv 1* gene product, is involved in multivalent repression of the isoleucine-valine enzymes, probably functioning as a positive effector. Altered repression of the isoleucine-valine biosynthetic enzymes was observed in *Saccharomyces cerevisiae* MAR-33 (ref. 10), with a mutation in or closely linked to the *ilv 1* gene which renders threonine deaminase 100 times less sensitive than the wild type to isoleucine inhibition^{11,12}. The isoleucine-valine biosynthetic enzymes from MAR-33 are fully derepressed when the strain is grown on minimal medium while normally only partial derepression is attained under these conditions^{11,12}. Altered regulation of the isoleucine-valine enzymes was observed also in strain D106-1a, which contains a nonsense mutation in the middle of the *ilv 1*

gene^{11,12}. In D106-1a, the isoleucine-valine biosynthetic enzymes fail to derepress when cells are grown in minimal medium or in a medium limited for isoleucine, a condition that normally leads to complete derepression. Magee and I have shown that the altered regulatory effects on the isoleucine-valine enzymes cosegregate with the desensitising threonine deaminase mutation in MAR-33 and the *ilv 1* nonsense mutation^{11,12}.

In vivo evidence for threonine deaminase or some form of the *ilv 1* gene product in multivalent repression of the isoleucine-valine pathway has been obtained also in bacteria in Umbarger's laboratory^{13,14} as well as in that of Hatfield¹⁵, who suggested

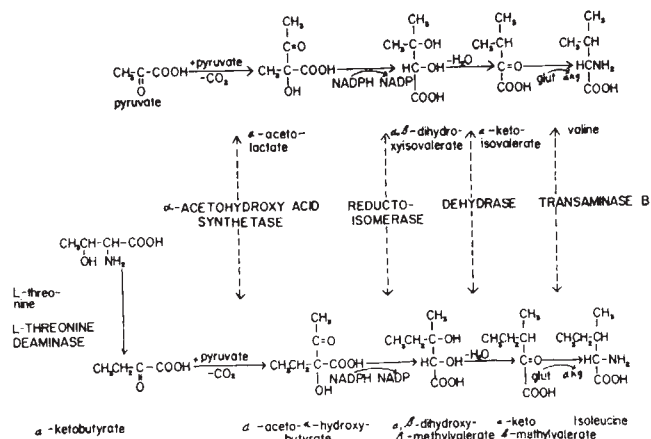


Fig. 1 Biosynthetic pathway for isoleucine and valine in *Saccharomyces cerevisiae*. Threonine deaminase (EC 4.2.1.16) is the first enzyme specific for the biosynthesis of isoleucine. The other four enzymes are involved in the biosynthesis of both valine and isoleucine. Repression of AHAS, reductoisomerase, dehydratase and transaminase B requires isoleucine, valine and leucine each at 5 mM. In *Saccharomyces cerevisiae*, the genes which code for the five different isoleucine-valine enzymes are on different chromosomes.

Table 1 List of strains containing *ilv 1* mutations

Genotype	Phenotype	Reference
<i>ilv 1-1</i>	Nonsense mutation	11
<i>ilv 1-8</i>	Nonsense mutation	11
<i>ilv 1-15</i>	Nonsuppressible	20
<i>ilv 1-41</i>	Nonsuppressible	20
<i>ilv 1-48</i>	Nonsuppressible	20
<i>ilv 1-51</i>	Nonsuppressible	20
<i>ilv 1-67</i>	Nonsense mutation	20
<i>ilv 1-74</i>	Nonsuppressible	20
<i>ilv 1-82</i>	Nonsuppressible	20
<i>ilv 1-83</i>	Nonsuppressible	20
<i>ilv 1-101</i>	Nonsense mutation	20
<i>ilv 1-113</i>	Nonsense mutation	20
<i>ilv 1-1 SUP, ilv 3-</i>	Contains suppressor for <i>ilv 1-1</i>	11
<i>ilv 1-101 SUP, ilv 3-</i>	Contains suppressor for <i>ilv 1-101</i> This study	
M21	Nonsuppressible <i>ilv 1</i>	11
MD11	Wild type for <i>ilv 1</i>	24

some role for threonine deaminase in repression¹⁶. In addition to the isoleucine-valine pathway, the involvement of a biosynthetic enzyme in repression of subsequent enzymes of the histidine pathway has been shown by Goldberger *et al.*^{17,18}.

The diversity of protein function became clearer with reports of proteins with several activities^{7,11,17}. I report here the analysis of the catalytic and regulatory functions of the *ilv 1* gene. I present evidence confirming the multifunctional nature of the *ilv 1* gene where by genetic and biochemical analyses, the intracistronic discrimination of some of the *ilv 1* regions associated with each function are assigned.

Catalytic function of *ilv 1*

Figure 1 shows one activity of the *ilv 1* gene product is the conversion of L-threonine to α -ketobutyrate, the first biosynthetic step leading to the synthesis of L-isoleucine. This catalytic activity is performed by threonine deaminase whose activity is 50% inhibited by 0.5 mM L-isoleucine and stimulated by 5 mM L-valine¹⁰. The threonine deaminase from *S. cerevisiae* contains two identical subunits of approximately 100,000 molecular weight^{10,19}.

Several strains of *S. cerevisiae* with mutations in the *ilv 1* gene, rendering threonine deaminase catalytically inactive²⁰, have been tested (Table 1). Each *ilv 1* allele represented in the table has been subjected to genetic fine structure analysis and the map is given in Fig. 2 (ref. 20). As Table 1 shows the *ilv 1* mutations 1, 101, 67, 117, 113 and 8 are adjudged to be nonsense mutations by suppression studies²⁰, whereas suppressibility of the other *ilv 1* mutations has not been observed²⁰.

As Zimmerman²⁰ indicated, the genetic length of the *ilv 1* gene using the *ilv 1-41* and *ilv 1-8* alleles as its extremities is about 16 map units of induced mitotic recombination. Based on previous data^{21,22} one unit of recombination represents approximately 130 nucleotides. By these calculations the *ilv 1* gene product involved in catalytic function would have a molecular weight of approximately 100,000 which is consistent with a dimeric protein of approximately 200,000 molecular weight, as indicated above.

Threonine deaminase activity was found in extracts of several diploid strains each containing different pairs of *ilv 1* alleles. Intragenic complementation between various *ilv 1* alleles has been found by Zimmermann *et al.*²³. The diploid strains constructed by mating different haploid strains each containing various *ilv 1* mutations, as well as a diploid strain containing two wild type *ilv 1* genes, are given in Table 2.

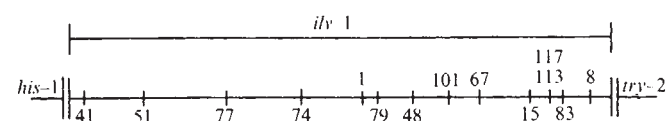


Fig. 2 Fine structure map of the *ilv 1* gene. The alleles above the line are of the nonsense type.

Extracts were prepared from the various diploid strains and the specific activity of threonine deaminase was determined as before^{10,12}. In addition, the sensitivity of the diploid threonine deaminase to inhibition by 1.0 mM and 100 mM L-isoleucine was determined. Table 2 shows threonine deaminase with a specific activity of 3.21 (μ mol of product formed per 20 min per mg of protein) was found in extracts from the diploid containing two wild type *ilv 1* genes. An unexpectedly high threonine deaminase specific activity of 1.23 was found for the diploid containing the *ilv 1-51* and *ilv 1-79* alleles, which is 40% of the specific activity found in the wild type diploid. Table 2 shows the threonine deaminase obtained from the diploid containing the *ilv 1-51* and *ilv 1-79* alleles was not inhibited by 1.0–100 mM L-isoleucine which inhibits *ilv 1+* $\times$ *ilv 1+* wild type threonine deaminase. As previously reported, 100 mM L-isoleucine inhibits the threonine deaminase obtained from strain MAR-33 harbouring a mutation in *ilv 1* (or closely linked to it) which renders the enzyme 100 times less sensitive to isoleucine inhibition. Table 2 shows of the other diploids tested for threonine deaminase activity, that containing the *ilv 1-48* and *ilv 1-77* alleles had a threonine deaminase specific activity of 0.31 which seemed to be sensitive to inhibition by 1.0 mM isoleucine. Neither the diploid containing the *ilv 1-48* and *ilv 1-82* alleles nor that containing the *ilv 1-51* and *ilv 1-82* alleles had detectable threonine deaminase activity.

Therefore, in addition to showing several strains containing *ilv 1* mutations rendering threonine deaminase either catalytically inactive or less sensitive to isoleucine inhibition, the use of diploids containing different *ilv 1* alleles has facilitated, by intragenic complementation, the *in vivo* construction of threonine deaminase molecules with altered properties for isoleucine inhibition.

Regulatory function of *ilv 1*

As previously indicated, in addition to catalytic activity, some form of the *ilv 1* gene product must be involved in multivalent repression of the isoleucine-valine biosynthetic enzymes,

Table 2 Threonine deaminase specific activity in diploid strains

Diploid genotype	L-isoleucine added (mM)		
	0	1.0	100
<i>ilv 1+ $\times$ ilv 1+</i>	3.21	0	0
<i>ilv 1-51 $\times$ ilv 1-79</i>	1.23	1.14	1.29
<i>ilv 1-48 $\times$ ilv 1-77</i>	0.31	0	0
<i>ilv 1-48 $\times$ ilv 1-82</i>	0		
<i>ilv 1-51 $\times$ ilv 1-83</i>	0		

The threonine deaminase was assayed as previously described¹⁰ in the absence of L-isoleucine and in the presence of 1.0 mM and 100 mM L-isoleucine. Specific activity is expressed as μ mol of α -ketobutyrate formed per 20 min per mg of protein. Diploid strains were grown in minimal medium and extracts were obtained for the threonine deaminase assay, as previously described^{10,12}.

probably acting as a positive effector^{11,12}. This function is designated the '*ilv 1* regulatory function'. Evidence for altered *ilv 1* regulatory function was sought in various strains containing different *ilv 1* mutations as well as in the diploid strains constructed by mating the various haploid *ilv 1* auxotrophs.

I analysed the *ilv 1* regulation function by two approaches. First, determinations were made of the acetohydroxy acid synthase (AHAS) specific activity obtained from the various *ilv 1* auxotrophs and diploid strains grown in minimal, repressing and isoleucine limiting medium (Table 3). Second, the AHAS differential rate of synthesis was determined in cultures which had been transferred from repressing medium to isoleucine limiting medium (that is kinetics of escape from repression).

Two major conclusions can be drawn from Table 3. First, the *ilv 1* regulatory function is altered in strains containing the *ilv 1* nonsense alleles *ilv 1-1* and *ilv 1-101*. Second, altered

Table 3 Effect of different *ilv 1* mutations on *ilv 1* regulatory function

<i>ilv-1</i> Mutation	Specific activity of AHAS			ILM* RM ratio
	Repressing medium	Minimal medium	Isoleucine limiting medium	
MD11	0.049 ± 0.013	0.147 ± 0.019		
M21	0.057 ± 0.014	0.139 ± 0.019	0.364 ± 0.029	6.40
<i>ilv 1-1</i> nonsense	0.069 ± 0.012	0.063 ± 0.015	0.067 ± 0.014	0.97
<i>ilv 1-8</i> nonsense	0.064 ± 0.014	0.133 ± 0.019	0.364 ± 0.032	5.70
<i>ilv 1-15</i>	0.061	0.131	0.351	5.75
<i>ilv 1-41</i>	0.041	0.110	0.282	6.88
<i>ilv 1-48</i>	0.064	0.131	0.315	4.93
<i>ilv 1-51</i>	0.059 ± 0.010	0.127 ± 0.020	0.341 ± 0.030	5.78
<i>ilv 1-67</i> nonsense	0.069 ± 0.015	0.140 ± 0.018	0.371 ± 0.035	5.38
<i>ilv 1-74</i>	0.056	0.132	0.322	5.75
<i>ilv 1-79</i>	0.063	0.125	0.341	5.41
<i>ilv 1-82</i>	0.056	0.124	0.311	5.55
<i>ilv 1-83</i>	0.073	0.141	0.348	4.77
<i>ilv 1-101</i> nonsense	0.062 ± 0.011	0.071 ± 0.013	0.069 ± 0.014	1.11
<i>ilv 1-113</i> nonsense	0.063 ± 0.013	0.128 ± 0.020	0.324 ± 0.030	5.14
<i>ilv 1-1 SUP, ilv 3-</i>	0.067 ± 0.013	0.134 ± 0.021	0.343 ± 0.029	5.11
<i>ilv 1-101 SUP, ilv 3-</i>	0.059 ± 0.014	0.121 ± 0.018	0.335 ± 0.033	5.67
<i>ilv 1+ × ilv 1+</i>	0.053 ± 0.011	0.135 ± 0.016		
<i>ilv 1-51 × ilv 1-79</i>	0.056 ± 0.013	0.281 ± 0.024		
<i>ilv 1-48 × ilv 1-77</i>	0.041 ± 0.010	0.143 ± 0.021		
<i>ilv 1-48 × ilv 1-82</i>	0.061	0.141		
<i>ilv 1-51 × ilv 1-83</i>	0.053	0.119		

The repressing medium used is minimal salts medium plus L-isoleucine, L-valine and L-leucine, each at 5 mM^{12,24}. Minimal medium consisted of minimal salts medium as previously described^{10,22}. Where isoleucine was necessary for growth, a nonrepressing concentration of 2 mM L-isoleucine was added^{12,24}. Isoleucine limiting medium consisted of minimal salts medium plus 2.5 mM L-isoleucine and 20 mM L-valine which competes for isoleucine utilisation^{12,24}. AHAS was assayed in toluenised cells as previously described^{12,24}. Its specific activity is expressed as μmol of product produced per 20 min per mg of protein. MD11 is a strain wild type for the *ilv 1* gene. M21 contains a nonsuppressible *ilv 1* mutation which renders threonine deaminase catalytically inactive. In the cases where standard deviations are represented, four determinations were done.

* Ratio of AHAS activity obtained from cells grown in isoleucine limiting medium (ILM) compared with cells grown in repressing medium (RM).

ilv 1 regulatory function is not observed in strains containing the *ilv 1* nonsense alleles, *ilv 1-67*, *ilv 1-113*, *ilv 1-117*, and *ilv 1-8*.

As Table 3 shows, AHAS was derepressed approximately 2.5-fold when the normally regulated strains MD11 and M21^{11,12} were grown on minimal medium compared with repressing medium: the ratio of AHAS specific activity of M21 cells grown in isoleucine limiting medium compared with repressing medium was 6.4. Similar results were obtained for all strains containing the various *ilv 1* alleles including the *ilv 1-67*, *ilv 1-113*, *ilv 1-117* and *ilv 1-8* nonsense alleles, with the exception of strains containing the *ilv 1-1* and *ilv 1-101* nonsense alleles where no AHAS derepression was observed when cells were grown in minimal or isoleucine limiting medium. The ratio of AHAS specific activity when strains containing either the *ilv 1-1* or *ilv 1-101* alleles were grown on isoleucine limiting medium compared with repressing medium was close to unity. Since the AHAS specific activity obtained from strains containing the *ilv 1-1* or *ilv 1-101* alleles grown in repressing medium was similar to that obtained for the normally regulated strains MD11 and M21, unity represents a failure to derepress

under minimal and isoleucine limiting conditions.

Since only the *ilv 1-1* and *ilv 1-101* nonsense mutations and not the *ilv 1-8*, *ilv 1-67*, *ilv 1-117* and *ilv 1-113* nonsense mutations affect the *ilv 1* regulatory function, one might conclude that the *ilv 1* region excluded by the *ilv 1-1* and *ilv 1-101* nonsense mutations are needed for regulatory function whereas the regions excluded by the other *ilv 1* nonsense mutations are not.

These results were substantiated by examining the differential rate of synthesis of AHAS by the second method in cultures transferred from repressing medium to isoleucine limiting medium^{11,12}. Figure 3 gives the differential rate of AHAS synthesis in M21, which is normally regulated for the isoleucine-valine enzymes—it was 0.19. The AHAS differential rate in the strain containing *ilv 1-1* was 0.017 and in the strain containing *ilv 1-101*, 0.021. As Table 4 shows AHAS differential rate of 0.02 was obtained when M21 was grown in repressing medium. The low AHAS differential rate obtained from the strains containing the *ilv 1-1* and *ilv 1-101* alleles grown on isoleucine limiting medium therefore, reflects lack of derepression. Also Table 4 shows the strains containing the other nonsense alleles, *ilv 1-113*, *ilv 1-8* and *ilv 1-67*, strains containing suppressors for the *ilv 1-1* and *ilv 1-101*, *ilv 1-1 SUP* and *ilv 1-101 SUP* and strains containing the nonsuppressible *ilv-1* alleles 51 and 79 gave AHAS differential rates of synthesis comparable with the normal value obtained with M21.

Figure 3 also shows that the increase in the differential rate of synthesis of AHAS was inhibited by 50 $\mu\text{g ml}^{-1}$ of cycloheximide, which inhibits protein synthesis. Thus the increase in activity required protein synthesis and probably represented synthesis of new enzyme. In addition, since the level of AHAS after inhibition of protein synthesis remained constant for 4 h, no substantial degradation of AHAS occurred within the 4 h period.

The altered *ilv 1* regulatory function and the *ilv 1-1* and *ilv 1-101* mutations cosegregate in four tetrads tested. As Table 3 shows, both catalytic and regulatory function was restored when suppressors for the *ilv 1-1* and *ilv 1-101* nonsense mutations were present. In addition, the altered *ilv 1* regulatory

Table 4 Differential rate of AHAS synthesis in *ilv 1* auxotrophs

<i>ilv</i> Mutation	Differential rate of synthesis of AHAS*
M21	0.190
<i>ilv 1-1</i>	0.017
<i>ilv 1-101</i>	0.021
<i>ilv 1-113</i>	0.160
<i>ilv 1-8</i>	0.205
<i>ilv 1-67</i>	0.170
<i>ilv 1-51</i>	0.168
<i>ilv 1-79</i>	0.210
<i>ilv 1-1 SUP ilv 3</i>	0.180
<i>ilv 1-101 SUP ilv 3</i>	0.230
(M21-grown in repressing medium)†	0.020

* μmol of product produced per 20 min per 10^6 cells.

† Differential rate of synthesis for AHAS when strain M21 is grown on repressing medium.

Strains were transferred from repressing medium to isoleucine limiting medium as described for Fig. 3.

function resulting from the *ilv 1-1* and *ilv 1-101* mutations was recessive to wild type (Table 5). When a strain containing either the *ilv 1-1* or *ilv 1-101* mutations was mated with a strain which was *ilv 1*⁺, *ilv 2*⁻, and the resultant diploid strain was grown on minimal medium, the AHAS specific activity was derepressed 2.5 times more than the normal repressed level. These results suggest that the *ilv 1* gene product is necessary for derepression of the isoleucine-valine enzymes.

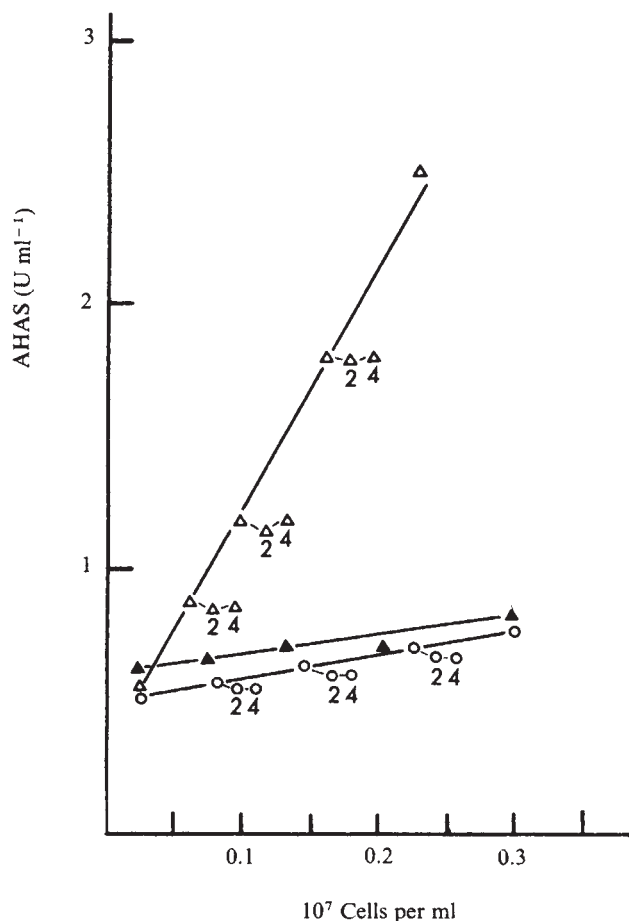


Fig. 3 Cells were grown in 50 ml of repressing medium to about 5×10^7 cells per ml, centrifuged, washed and diluted five times into 250 ml of minimal medium plus 20 mM valine and 2.5 mM isoleucine (isoleucine limiting medium). Samples of 50 ml were taken after 0, 2, 4, 6 and 8 h. Cells were made permeable by toluene and the AHAS was assayed as previously reported^{12,24}. The numbers 2 and 4 shown after each point represent AHAS activity 2 and 4 h after the addition of 50 μ g of cycloheximide. Open triangles represent strain M21, closed triangles represent the strain containing the *ilv 1-1* allele and the open circles represent the strain containing the *ilv 1-101* allele.

There is evidence^{11,12} that the isoleucine inhibition property of threonine deaminase is related to the *ilv 1* regulatory function. In a strain MAR-33, which contains a mutation in (or closely linked to *ilv 1*), the threonine deaminase is 100 times less sensitive to isoleucine inhibition¹⁰. When MAR-33 was grown on minimal medium, the AHAS as well as the other isoleucine-valine enzymes were derepressed approximately six-fold, attaining a specific activity usually obtained only under isoleucine limiting conditions. If 5 mM L-isoleucine was added to the medium, the isoleucine-valine enzymes were derepressed only 2.5-fold which is usually attained when a wild type strain is grown on minimal medium^{11,12}. In another strain TIR-9 which also contains a mutation in *ilv 1* (or closely linked to it), the threonine deaminase is only ten times less

sensitive to isoleucine inhibition¹⁰. The levels of the isoleucine-valine enzymes when TIR-9 was grown under minimal conditions were like wild type. Therefore, it seems that a 100-fold but not a ten-fold isoleucine desensitisation of the threonine deaminase affects the *ilv 1* regulatory function.

Table 5 Recessivity of altered *ilv 1* regulatory function

Medium	Diploid genotype	Specific activity of AHAS
Minimal	<i>ilv 1-1 ilv 2</i> ⁺ $\times$ <i>ilv 1</i> ⁺ <i>ilv 2</i> ⁻	0.123 ± 0.017
	<i>ilv 1-101 ilv 2</i> ⁺ $\times$ <i>ilv 1</i> ⁺ <i>ilv 2</i> ⁻	0.112 ± 0.019
Repressing	<i>ilv 1-1 ilv 2</i> ⁺ $\times$ <i>ilv 1</i> ⁺ <i>ilv 2</i> ⁻	0.041 ± 0.011
	<i>ilv 1-101 ilv 2</i> ⁺ $\times$ <i>ilv 1</i> ⁺ <i>ilv 2</i> ⁻	0.047 ± 0.010

AHAS was assayed as previously described^{12,24}, and specific activities are expressed as μ mol of product produced per 20 min per mg of protein.

The *ilv 1* regulatory function was examined in the various diploid strains previously described, especially that which contains the *ilv 1-51* and *ilv 1-79* alleles which result in threonine deaminase desensitised to isoleucine inhibition (Table 3). In all diploids tested except one, the AHAS-specific activity was approximately 2.5 times greater when cells were grown in minimal medium compared with repressing medium. In the diploids strain containing the *ilv 1-51* and *ilv 1-79* alleles, the AHAS specific activity was five times greater when cells were grown in minimal media compared with repressing media. This increased derepression of the AHAS when the diploid strain containing *ilv 1-51* and *ilv 1-79* alleles was grown on minimal media further indicates the interrelationship of the isoleucine inhibition site of the *ilv 1* product, and the *ilv 1* regulatory function.

Multifunctional map of *ilv 1*

A summary of the effects of the various *ilv 1* mutations on *ilv 1* catalytic and regulatory function is represented in Fig. 4. The results are represented as a multifunctional map which consist of three representations of the *ilv 1* gene each corresponding to a different property of the *ilv 1* gene product and each containing the *ilv 1* alleles causing defects in the corresponding *ilv 1* function.

As Fig. 4 shows, the *ilv 1-1* and *ilv 1-101* nonsense alleles concordantly affect both catalytic and regulatory function, whereas both functions are discordantly affected by the *ilv 1-113*, *ilv 1-117*, *ilv 1-67*, and *ilv 1-8* nonsense alleles where only the catalytic function is affected. In addition, the other nonsuppressible *ilv 1* alleles did not affect *ilv 1* regulatory function.

These results suggest that the *ilv 1* regions affected by the *ilv 1-117*, *ilv 1-113*, *ilv 1-67* and *ilv 1-8* nonsense mutations are not needed for regulatory function but are necessary for catalytic function. On the contrary, *ilv 1-1* and *ilv 1-101* nonsense mutations affect *ilv 1* regions needed for both regulatory and catalytic functions. In addition, these results represent the strongest indication that the regulatory function actually is within the *ilv 1* gene and not in an adjacent regulatory region.

In addition, as seen in Fig. 4, alleles *ilv 1-51* and *ilv 1-79* do not affect *ilv 1* regulatory function in haploid strains. Nevertheless, regulatory function is altered in a diploid strain containing both *ilv 1-51* and *ilv 1-79* alleles, where intragenic complementation results in restoration of threonine deaminase activity that is insensitive to isoleucine inhibition. These results are consistent with the report of a concordant effect on isoleucine inhibition and regulatory function in strain MAR-33 (refs 11, 12) Preliminary data indicate that MAR-33 has a

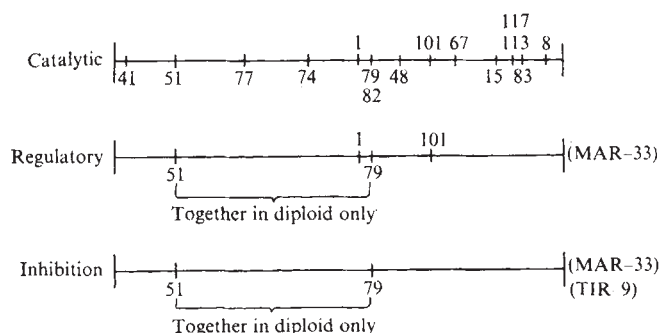


Fig. 4 Multifunctional map of the *ilv 1* gene. Each representation of the *ilv 1* gene corresponds to one of the *ilv 1* functions which are represented at the left of the corresponding cistron. Only the alleles which result in altered function are represented. The alleles above the line are of the nonsense type.

mutation near the middle of the *ilv 1* gene and that it is not suppressible (my unpublished data).

In conclusion, the results presented here strongly indicate that the *ilv 1* gene is multifunctional, coding for a product which functions in the catalysis of L-threonine to α -keto-butyrate, as well as being a positive effector in the derepression of the isoleucine-valine enzymes. I have found correlation between the isoleucine inhibition property of threonine deaminase and *ilv 1* regulatory function. Furthermore, there is intracistronic discrimination of the *ilv 1* catalytic and regulatory functions. Apparently the regulatory function of the multifunctional *ilv 1* gene represents an example of a eukaryotic regulatory gene which has been identified and partially characterised both genetically and biochemically.

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letters to nature

Cosmogony of the asteroidal belt

We have to derive the state of partial corotation in a way which, by being very simple, stresses its character as a basic state in cosmic plasma dynamics. We also substitute the mass density (m , a) for the number density (n , a) in the plot of asteroids, as a function of their semimajor axis a . This gives a better definition to the limits of the main belt, and demonstrates more clearly that the asteroids—like the Saturnian rings—have condensed from a partially corotating plasma.

If a rotating body has an axisymmetric dipole field and is surrounded by a plasma, and if the electrical conductivity of the body and of the plasma is infinite, the plasma will rotate with the same angular velocity Ω as the body; the magnetic field lines are 'frozen-in'. This idealised model is, however, not applicable to cosmic problems because celestial bodies have gravitation, and the plasma is subject both to that and to the centrifugal force produced by the rotation¹⁻⁴. Moreover, the 'frozen-in' picture is seldom applicable in astrophysics⁴⁻⁷. The assumption of infinite conductivity is unrealistic, because electric fields parallel to the magnetic field play an important role^{8,9}. It is now evident that the magnetosphere is to a high degree uncoupled from the ionosphere¹⁰.

This means that instead of a plasma corotating with an angular velocity $\omega = \Omega$, we have typically a 'partial corotation' with $\omega < \Omega$ (refs 3 and 4). The rotational velocity is given by the condition that the centrifugal force, f_c , the gravitation, f_g , and the electromagnetic force, f_B , acting on a plasma element should balance each other, so that

$$f_c + f_g + f_B = 0 \quad (1)$$

(We assume that the plasma temperature is so low that pressure and diamagnetic effects can be neglected—see Fig. 1.) If M_c is the mass of the central body, κ the constant of gravitation, B the magnetic field deriving from a dipole with moment a , m the mass of a plasma element, I the current through it, and x_0 a unit vector perpendicular to the axis, there is in a coordinate system (r, λ) in the meridian plane of the dipole:

$$f_c = \omega^2 m r \cos \lambda x_0 \quad (2)$$

$$f_g = (\eta M_c m / r^3)(-r) \quad (3)$$

$$f_B = I \times B \quad (4)$$

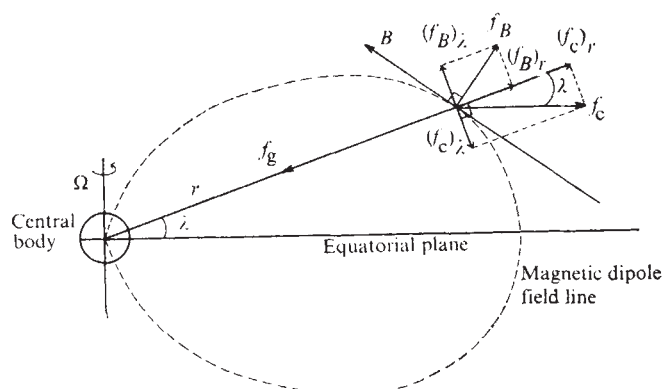


Fig. 1 Configuration of partial corotation. Equilibrium between gravitation f_g , centrifugal force f_c , and electromagnetic force $f_B = \mathbf{I} \times \mathbf{B}$, means that $f_g + f_c + f_B = 0$. Because $(f_c)_\lambda + (f_B)_\lambda = 0$, the geometry of the dipole field requires that $(f_c)_r = 2(f_B)_r = 2/3(-f_g)_r$. a, Central body; b, magnetic dipole field line.

In a dipole field $B_r = -(2a/r^3) \sin \lambda$, $B_\lambda = (a/r^3) \cos \lambda$, and so from equations (1)–(4) (for $\lambda \neq 0$)

$$f_c = 2al/r^3$$

and

$$f_g = [f_c + (la/r^3)] \cos \lambda$$

showing that: $(f_c)_r = 2(f_B)_r = (2/3) |f_g|$. Thus the gravitational force is compensated to 2/3 by the centrifugal force, and to 1/3 by the electromagnetic force. The factor 2/3 is given by the geometry of a dipole field.

If condensation of grains (planetesimals) takes place from a partially corotating plasma, and the resulting grains are so large that the electromagnetic forces become small compared to f_c and f_g , then they will move in Kepler orbits. As the centrifugal force does not fully compensate the gravitation, the condensed grains will move in ellipses with eccentricity, $e = 1/3$ (refs 1–4). If a large number of grains have condensed in the neighbourhood of the central body and they collide with one another, the eccentricities of their orbits will diminish, so that eventually they will all move in more circular orbits, with a semimajor axis with a length which is 2/3 the distance to the point where the condensation occurred.

There are reasons to believe that condensation from a partially corotating plasma was a basic process in the formation of the Solar System. In fact, the structure of the Saturnian ring, and of the asteroid belt can be understood in this way^{1–3,11}. The factor 2/3 occurs in two places in the Saturnian ring system, and in two places in the asteroid belt. In the latter case the usual (n, a) diagram of asteroids has been used, giving the number density, n , of asteroids as a function of their semimajor axes, a .

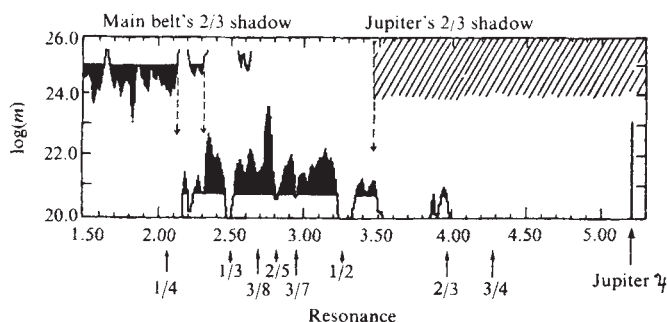


Fig. 2 The smoothed (m, a) diagram. The curve has been smoothed with the weight function 1:3:5:3:1. Mass distribution in unit of gram within a radial distance interval of $\Delta a = 0.01$ AU. In order to emphasise the log scale, high density regions are darkened. The same diagram diminished by a factor 2/3 and turned upside down is shown above, in order to demonstrate the 'shadow' effect which produces the inner cutoff of the asteroidal belt. Similarly Jupiter's 'shadow', which generates the outer cutoff, is shown. Kirkwood gaps from Jupiter resonances are arrowed.

The diagram (n, a) only gives an approximate picture of the mass distribution in the asteroid belt. We have now, however, calculated an (m, a) diagram, giving the mass distribution. We have assumed that all asteroids have the same albedo and density, so that we can put their mass proportional to $(\text{brightness})^{3/2}$ (Fig. 2).

The (m, a) diagram shows the Kirkwood gaps, especially at 1/2 and 1/3, somewhat more clearly than the usual (n, a) diagram.

The limits of the main belt at $a = 2.15$ and 3.49 are also sharper (especially because a large number of asteroids below $a = 2.15$ have a negligible mass).

In order to study the effect of the fall-down ratio, 2/3, the (m, a) diagram, diminished by a factor 2/3, is shown reversed in the upper part of Fig. 2.

The diagram shows that the whole main belt is located below 2/3 of Jupiter's orbit, which means that the main belt asteroids may have derived from condensation which took place inside Jupiter's orbit down to $a = 3.49$. At this limit plasma would have condensed directly on already existing asteroids (or asteroidal grains), which therefore produced a 'shadow' at 2/3 this distance. As the diagram shows, there is a marked drop in mass density at $2/3 \times 3.49 = 2.32$. Above the 1:2 Kirkwood gap however, the mass density does not seem to be large enough to make the plasma density drop to zero. That does not occur until the 'shadow' of the bulk of asteroids below 3.20 appears at 2.13 (that is, $2/3 \times 3.20$).

There is no other known way to explain the upper and lower limits of the main belt.

The only asteroids outside the main belt which are massive enough to appear in the diagram are the Hildas, at $a = 3.95$ AU. As 5.9 AU ($3/2 \times 3.95$) exceeds Jupiter's distance from the Sun ($= 5.2$) they must have condensed outside Jupiter's orbit. When falling down in ellipses with $e = 1/3$ they avoided capture (or dispersal) by Jupiter because they were commensurable with Jupiter's orbit period to a degree of 2/3 that kept them from coming close to Jupiter. (Compare the Neptune–Pluto resonance and similar cases¹²).

The 2/3 ratio is found in three places in the asteroid (m, a) plot (Fig. 2). In fact, the observed ratios agree with the theoretical value to within 1%. Including the two occurrences in the Saturnian ring system there are five cases which confirm that the condensation from a plasma in partial corotation was an important process in the formation of the Solar System.

The mass distribution of the asteroids is not altogether explained by the resonances and the 2/3 fall-down. For example, there is a very strong peak at 2.75 which includes Ceres and Pallas, and a secondary peak at 2.36 which includes Vesta. These may result from a later evolution which concentrates mass through a process which eventually leads to the formation of planets.

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A Lagrangian community?

It seems that it may be economically feasible, within the limits of the technology of this decade, to establish at one of the Lagrange libration points of the Earth-Moon system (called here L_5) a habitat capable of supporting and maintaining some 10,000 people. Projections indicate that this habitat, using free solar energy and the rich mineral resources of the lunar surface, could construct a still larger habitat, in a progression leading, possibly within 30 yr from now, to communities of from 10^5 to 10^7 people. These communities could be as comfortable as the most desirable parts of the Earth, with natural sunshine, controlled weather, normal air, apparent gravity and complete freedom from pollution. Replication of these communities could lead to the exponential growth of new land area, with a growth rate more rapid than that of the total human population.

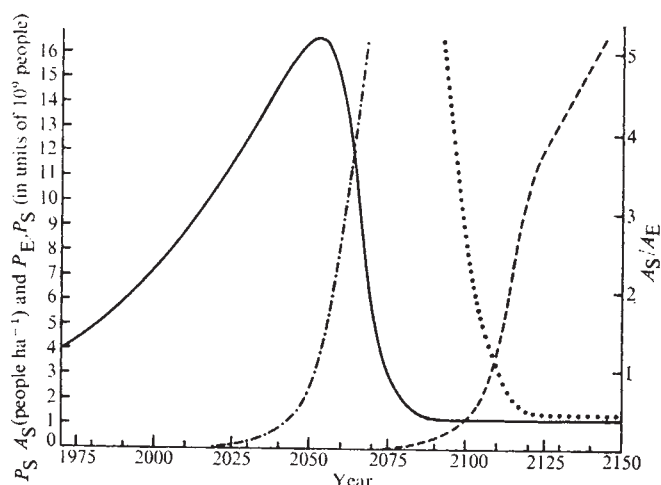


Fig. 1 A graph, technically realisable in my opinion, showing that most industry could be removed from the biosphere of the Earth within a century. The graph is based on a "worst-case" assumption: no reduction of the population growth rate either on earth or in the space communities. P_E is the population on earth, P_S the population in space, A_S/A_E the ratio of land area in space (all usable) to the total land area of Earth. Changes within wide limits in the assumed input numbers do not affect reaching a solution stable in P_E and P_S/A_S . The final stable value of P_E (1.2×10^9 people) is equivalent to the 1910 value. —, P_E ; ---, P_S ;, P_S/A_S ; ---, A_S/A_E .

A technically possible time-development is shown in Fig. 1, not as a prediction but as an illustration of the power of the technique.

Economic feasibility is defined here as the achievement of an overall cost less than or equal to that of the Apollo project. A reduction in the design size of the first habitat, with a corresponding reduction in the size of its population, would result in some further reduction in the estimates.

The solutions to several problems were necessary in order to achieve this feasibility:

(a) Geometry: A physical arrangement has been found which would allow the use of natural sunlight for industry, farming and the maintenance of an attractive, earthlike environment with normal day/night and seasonal cycles.

(b) Transport from the earth to L_5 : A reconfiguration of hardware already under development for the space-shuttle (to be operational by 1980) seems adequate to transport the necessary minimum of materials from earth.

(c) Water: The combination, at L_5 , of hydrogen from the earth with oxygen from the abundant oxides of the lunar surface effects an important saving of a factor 9 in the mass of material needed from earth per unit mass of water at L_5 .

(d) Materials: Obtaining nearly all the mass of the habitat from the moon appears essential for economy. Two alternative designs have been studied for the acceleration of lunar materials to the 2.4 km s^{-1} escape velocity of the moon. Both designs depend on the vacuum environment of the moon. Neither is conventional, but the technology of the present decade would suffice for either.

The ultimate benefits of this new possibility depend on the production of successively larger communities by a workforce which is housed, fed and maintained within the communities rather than on earth, and so to the eventual achievement by the communities of the ability to sustain their own growth by the construction of new lands and production facilities from lunar and asteroidal material.

These studies have been discussed in lectures at a number of universities during the past 18 months. Knowledge of the work has therefore spread, additional people have joined the effort, and recent progress has been rapid. On May 10, 1974, the first public meeting on this topic was held at Princeton University.

Detailed information on these studies will be found in forthcoming publications in *Physics Today* and in *Icarus*.

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Intensity of the near infrared OH airglow

THE OH airglow was studied from the balloon-borne telescope Thisbe on three flights launched in 1971 and 1972 from the NCAR balloon flight station, Palestine, Texas (32°N , 95°W). The principal goal of these flights has been the measurement of the zodiacal light in the near infrared¹ with the airglow as a troublesome foreground contribution which had to be determined carefully.

The measurements have been performed from a float altitude of 32 km at wavelengths $\lambda = 0.82$, 2.1, and $2.4 \mu\text{m}$ with half power bandwidths $\Delta\lambda = 0.023$, 0.1 and $0.1 \mu\text{m}$ respectively. For the shorter wavelengths a straightforward photometer with a multiplier as detector has been used. The larger wavelengths were measured by a dry ice cooled PbS-photometer. The field of view of both photometers was 2° in diameter. The $0.82 \mu\text{m}$ photometer was calibrated by observations of 33 stars. The PbS-photometer was calibrated before the flight by a 240 K blackbody and during the flight by the star $\alpha\text{-Ori}$

$$(I_{2.1 \mu\text{m}} = 1.9 \times 10^{-12} \text{ W cm}^{-2} \mu\text{m}^{-1}; \\ I_{2.4 \mu\text{m}} = 1.3 \times 10^{-12} \text{ W cm}^{-2} \mu\text{m}^{-1}).$$

The airglow intensity was determined from several elevation scans. As an example Fig. 1 shows the result of an elevation scan at $\lambda = 2.1 \mu\text{m}$.

A van Rhijn curve for an emission altitude of 92 km was fitted to the data. The resulting zenith intensities at all measured wavelengths are listed in Table 1.

Table 1 Zenith intensities of the OH airglow ($\text{W cm}^{-2} \text{sr}^{-1} \mu\text{m}^{-1}$)

Date	Time (CST)	λ (μm)	$\Delta\lambda$ (μm)	Intensity
May 13, 1972	23:00	0.82	0.023	$(2.5 \pm 0.4) \times 10^{-10}$
November 1, 1971	2:40	2.1	0.1	$(1.6 \pm 0.5) \times 10^{-9}$
October 24, 1972	2:00	2.1	0.1	$(1.6 \pm 0.3) \times 10^{-9}$
October 24, 1972	5:00	2.4	0.1	$< 6 \times 10^{-11}$

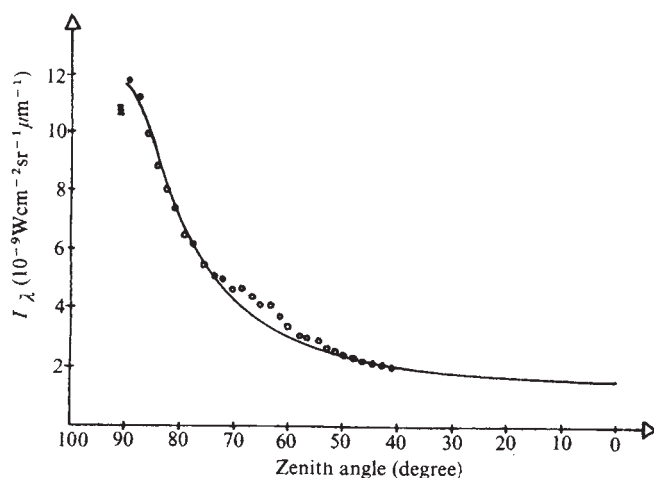


Fig. 1 OH airglow intensity at $2.1 \mu\text{m}$ determined from an elevation scan. A van Rhijn curve for an emission altitude of 92 km is fitted to the data. Notice the patchy structure at 65° zenith angle. $\lambda = 2.1 \mu\text{m}$; $\Delta\lambda = 0.1 \mu\text{m}$.

For comparison the results of other investigators are given. Sternberg and Ingham² found an average intensity of $2.9 \times 10^{-10} \text{ W cm}^{-2} \text{sr}^{-1} \mu\text{m}^{-1}$ at $\lambda = 0.82 \mu\text{m}$. At $\lambda = 2.1 \mu\text{m}$ Noxon *et al.*³ determined an intensity of 1×10^{-8} and Moros⁴ an intensity of $5 \times 10^{-10} \text{ W cm}^{-2} \text{sr}^{-1} \mu\text{m}^{-1}$. Peterson and Kieffaber⁵ determined at $\lambda = 2.2 \mu\text{m}$ ($\Delta\lambda = 0.54 \mu\text{m}$) an airglow intensity of $3.2 \times 10^{-9} \text{ W cm}^{-2} \text{sr}^{-1} \mu\text{m}^{-1}$ from a broadband measurement. The differences of the results may be

explained by the local and temporal variations of the airglow intensity. At $\lambda = 2.4 \mu\text{m}$ Moros⁴ found an upper limit of $< 2 \times 10^{-10}$ and Noxon and Vallance Jones⁶ an upper limit $< 10^{-8} \text{ W cm}^{-2} \text{sr}^{-1} \mu\text{m}^{-1}$. Our measurements indicate that at $2.4 \mu\text{m}$ the airglow intensity is at least three times smaller than previously known.

The patchy structure of the airglow was detected at 0.82 and $2.1 \mu\text{m}$ (see Figs 2 and 1). The patches have diameters of about 10° to 20° corresponding to 20 to 40 km in linear size. They differ from the average intensity up to 20% . Patches were also found at $0.8 \mu\text{m}$ by Peterson⁷ and at $2.2 \mu\text{m}$ by Peterson and Kieffaber⁵ using ground based measurements.

Our results have confirmed the existence of a theoretically predicted gap in the OH airglow emission at $\lambda = 2.4 \mu\text{m}$, which is also indicated in spectroscopic work^{8,9}. So surface photometry of extended celestial sources as the zodiacal light and the Milky Way is possible within this $\sim 0.3 \mu\text{m}$ wide gap from balloon altitudes. Because of the large intensity and irregularities of the OH airglow at shorter wavelengths serious problems arise in subtracting the airglow foreground from the night sky radiation.

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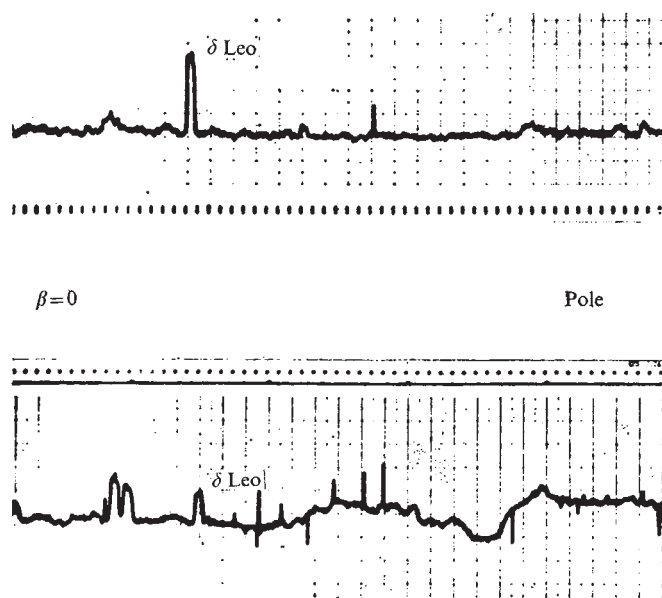


Fig. 2 Simultaneous scans from the ecliptic plane ($\beta = 0$) to the north celestial pole ($5,000 \text{ \AA}$ above, $8,200 \text{ \AA}$ below). Visual magnitude of the star $\delta \text{ Leo}$ ($A 4 \text{ V}$) is 2.56 mag . The signal registered at $8,200 \text{ \AA}$ is dominated by strong variations of the OH-airglow, at $5,000 \text{ \AA}$ the airglow contribution is much smaller.

Positron annihilation in EAS and ball lightning

CRAWFORD¹ has suggested that the bursts of γ -ray energy that correspond to positron annihilation at rest (0.511 MeV), which have been observed by Ashby and Whitehead², may be caused by extensive air showers (EAS) of primary energy $E_0 \approx 10^{16} \text{ eV}$. We present two major objections to that hypothesis.

First, we shall discuss the duration of the four γ -ray bursts which were detected. Ashby and Whitehead² report an upper limit of about 10 s for the duration of three events, and the fourth lasted about 3 s . Those two values corresponded to two different time constants of the recording device: 25 s and 1 s , respectively. The lower limit for the duration can be derived from the time resolution of the NaI(Tl) γ -ray detector which was used, about $0.25 \mu\text{s}$. A lower limit of $3.7 \times 10^3 \times 2.5 \times 10^{-7}$, $\approx 1 \text{ ms}$, can be given for the duration of the four events, as the event with the lower flux corresponds to 3.7×10^3 distinct counts. On the other hand, the delays in the distribution of the various EAS components must be considered. The electronic component, at distances relatively close to the shower axis, shows time-spreads of less than a few tens of ns (ref. 3), where-

as the delays observed for muons are of the order of several hundreds of ns. Slow neutrons which are delayed by no more than several μ s are the most delayed particles detected⁴. Finally, considering that positron annihilation times which correspond to the slower annihilation modes are of the order $\approx 10^{-7}$ s (ref. 5), it is hard to understand a γ -ray emission of 0.511 MeV from an EAS of such a long duration (≈ 1 ms.)

The number of photons which it is claimed were created seems rather high. Consider the energy deposited by the shower particles in the first radiation length in the ground, inside a disk of radius $R = 2$ m. The energy dissipated by an EAS is represented by the track length integral (ref. 6). In the lower atmosphere, after the maximum longitudinal development, the integral is represented by

$$E_{\text{diss}} = \varepsilon_0 N_{s,1} \int_{t_{\text{max}}}^{t_{\infty}} \exp [0.18 (t_{s,1} - t)] dt$$

where ε_0 is the 'critical energy' and its actual value in air is approximately 84 MeV. For a shower generated by a particle of energy $E_0 = 10^{16}$ eV, we assume $t_{\text{max}} = 18.6$, $t_{s,1} = 28.4$, and $t_{\infty} = 74.4$, considering t_{∞} when the EAS 'age parameter', s , is 2. The integral over the portion of the curve of the longitudinal development which is extrapolated below sea level (s.l.) to t_{∞} gives $E_{\text{diss}}/N_{s,1} = 465$ MeV, and the value of the same quantity for the first radiation length in the ground turns out to be 71 MeV. The quantity $N_{s,1}$ represents the size, at sea level, of a shower initiated by a particle of energy $E_0 = 10^{16}$ eV. On average it is 3×10^6 (ref. 7). Furthermore, we have to consider the lateral distribution of these particles to estimate the energy dissipated within the 2 m disk. Near the shower axis the density of particles, D , is well represented by the expression

$$D = N_{s,1}/500r$$

so that the number of particles inside the disk turns out to be approximately 2.5% of the total number. Thus, the energy dissipated in the first radiation length in the ground and in the region with $r = 2$ m will be

$$E_{\text{diss}}(r \leq 2 \text{ m}) = 2.5 \times 10^{-2} \times 3 \times 10^6 \times 71 \times 10^6 = 5.3 \times 10^{12} \text{ eV}$$

Assuming¹ that 5.5% of E_{diss} will be converted into photons with an energy of 0.511 MeV, a total of $5.7 \times 10^6 \gamma$ will be emitted. The detector has an estimated geometric factor of 3.6×10^{-4} so that about 210 photons are finally seen. That is an order of magnitude smaller than the lower value of γ detected in the four observed events.

To increase this value, a higher primary energy, E_0 , of more than 10^{17} eV is required. In this case, however, the flux of cosmic rays, Φ , is $2 \times 10^{-10} \text{ m}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$ (ref. 8). Thus, with a sensitive area, A of 10 m^2 , and a solid angle of acceptance, Ω of 3.1 sr, and assuming a running time, T , of 1.3×10^7 s, the number of events which can be expected is

$$n = \Phi A \Omega T = 0.08$$

In fact, four were observed under the same conditions.

Therefore, it seems to us that the hypothesis of γ -ray production from positrons of an EAS annihilating at rest has to be ruled out.

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Observations from Skylab of mesoscale turbulence in ocean currents

PHOTOGRAPHS and visual observations made by astronauts aboard Skylab have revealed remarkable eddies embedded in warm-water currents flowing poleward from equatorial oceans. These visual displays of mesoscale turbulence provide us with (1) a concept of the size and shape of such features, (2) an opportunity to examine the places of origin, (3) a feel for the rate at which they coalesce, and grow downstream, and (4) the opportunity to ascertain their significance in the thermal energy regime of the oceans.

I identified the eddies from photographs taken aboard Skylab 2 (May-June 73) over the northwest Caribbean Sea. Lying in the current, on the left-hand side, the vortices varied in diameter from 3-20 nautical miles. The associated atmospheric manifestations indicated cool surface water temperatures in the eddies, and down-current boundaries with waters warmer than those of the open current. There were (1) a smoother sea surface within the eddies than out-

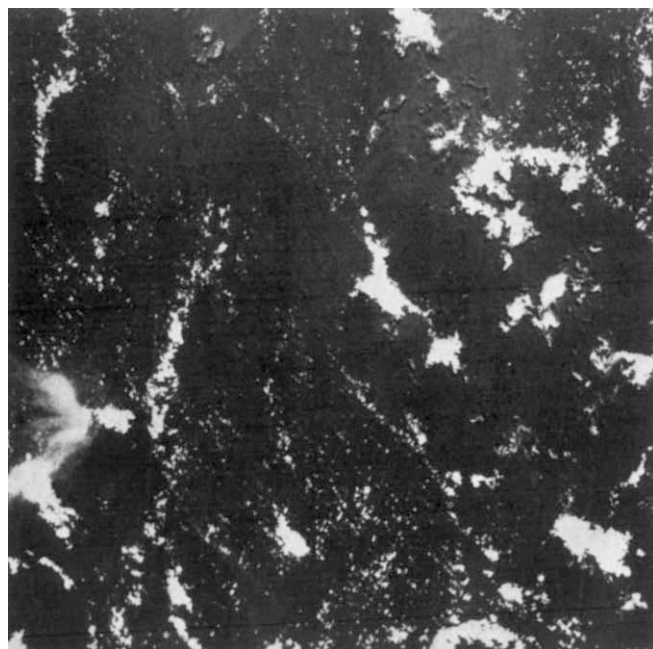


Fig. 1 Photograph (NASA No. SL2-10-072), taken vertically from the colour band of the Itek, 6-lens, 70 mm camera, with a 6 inch focal length lens, aboard the Skylab Space Station in June, 1973. The centre point of the area photographed is about $19^\circ \text{ N } 85^\circ \text{ W}$ and the ocean area covered is 60×60 nautical miles ($3,600 \text{ nm}^2$). Winds were from the southeast at 15-20 knots; the white, small "puff-ball" cumulus being aligned with the wind. The Yucatan Current flows north (top of picture) at speeds of 1-1.5 knots in this location, during this month of the year. The sea surface expression of the eddies are the oval, smoother, apparently indented features, and the atmospheric manifestations are the (1) disruption of the cloud streets, and (2) alignment of towering cumulus (to 35,000 feet) on the downstream edge of the eddies.

side, (2) a disruption of the wind-driven Langmuir circulation in both the sea surface and the overlying atmosphere, (3) towering crescent-shaped cumulus over the down current boundary, and (4) clear skies over the eddies (Fig. 1).

Although turbulent eddies of this nature and size have not been well documented, Spilhaus¹ noted eddies 30 miles in diameter during tests of the mechanical bathythermograph in the Gulf Stream. He pointed out that such scales

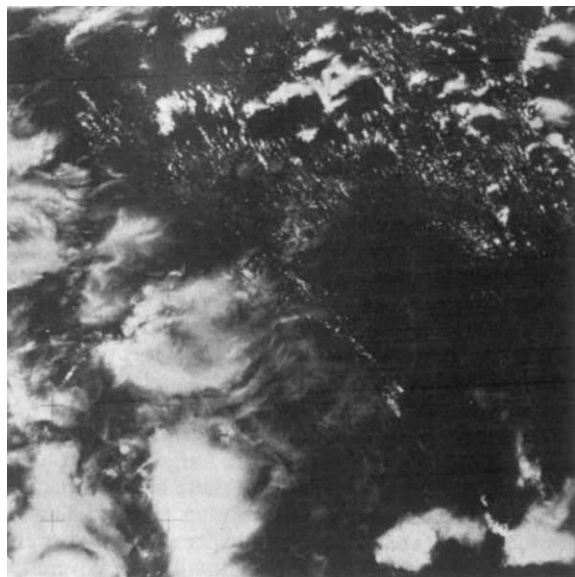


Fig. 2 Photograph (NASA No. SL4-137-3608) taken with a handheld Hasselblad, 70 mm camera, 100 mm focal length, from the Skylab Space Station, December, 1973, of the Southwest Atlantic Ocean near the coast of Argentina; Puerto Deseado was visible through the cumulus, middle left in the photograph. The centre point of the ocean covered by this photo is about $48^{\circ} 30' S$ $63^{\circ} W$ and the area is 210×210 nm ($44,400$ nm²). During this time of the year, upwelling takes place adjacent to the coast (beneath the huge, white cumulus caught in this photo) whereas about 100 miles offshore, a flow of warm water (50 – $55^{\circ} F$) moves south. Farther offshore, the cold (44 – $45^{\circ} F$) Falkland Current intrudes northward to the latitude of Montevideo ($35^{\circ} S$). On the day this photo was taken, the warm, nearshore counter-current was seen to be defined by the "puff-ball" cloud streets in the overlying marine atmosphere, into which intruded the clear skies overlying the cold eddies. The strong interaction between the countercurrent and the Falkland Current was manifested on this day by the strong blooms of phytoplankton; the light-coloured waters visible beneath the cumulus clouds.

implied a high rate of shear and were of the order of magnitude estimated by Rossby on the basis of a $10^{-3} s^{-1}$ shear. Further notations of "cold-water eddies"^{2,3} have been of eddies on current boundaries; formed from meanders, and are thus of a differing origin. That such turbulence has gone without much documentation is probably because of the (1) difficulty of detecting them by conventional oceanographic techniques, (2) a lack of knowledge of the sizes, persistence, and continuity of such features, and (3) the lack of photographs from orbital platforms which enhance the ocean/atmosphere manifestations of the eddies.

Because of their distinctive features, it was believed that the eddies could be examined visually by the three astronauts (Lt Col. Gerald Carr, Lt Col. William Pogue, and Dr Edward Gibson) aboard Skylab 4. Consequently, a request was made for visual observations by the astronauts early on the morning of 6 December 1973, as the spacecraft crossed the Caribbean Sea.

The characteristic clouds were indeed seen by all three astronauts, but the Sun angle was too low to enable examination of the ocean surface in the Sun's reflection. The next day, during a parallel orbital pass, but 180 miles to

the east, television on board scanned the Caribbean Sea, and the crescent-shaped clouds associated with the eddies were monitored to the coast of Columbia. Distinct eddies had also been caught by the camera during Skylab 3 (August–September), just south of those interpreted from Skylab 2. Similar cloud arrangements were also photographed south of Western Samoa, and the Gulf Stream east of Hatteras. The evidence was convincing, therefore, that turbulent vortices were constantly embedded within the main stream of warm ocean currents, and that they could be visually monitored from space.

Subsequently, observations from Skylab 4 confirmed that the turbulent features were in four other ocean areas (Coral Sea, North and South Equatorial Pacific, and western south Atlantic). Furthermore, eddies with similar surface manifestations, but not the towering cumulus, were observed east of New Zealand and in the Pacific north of Hawaii. These latter observations were confirmation that turbulent vortices of such magnitude are present wherever conditions are suitable, regardless of water temperature, and that eddies in warm currents are simply easier to see (and photograph) because of the atmospheric manifestations (Fig. 2).

We still had no definitive data, however, from the Caribbean Sea. Consequently, on January 24, 1974, personnel from the Navy's Weather Reconnaissance Squadron Four flew over the northwest Caribbean Sea, planting air expendable bathythermographs (AXBTs) along two lines, each about 200 miles long. An AXBT was dropped every ten nautical miles on both tracks. Heading south, the aircraft followed a flight line directly over waters on the left side of the core of the Caribbean Current. The second line was 60 miles to the east over the axis of the current.

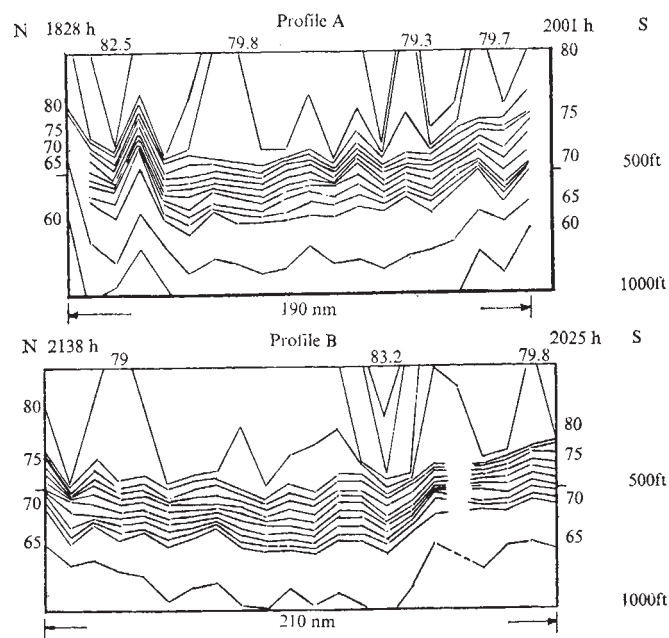


Fig. 3 North and south profiles of the vertical distribution of water temperature in the Yucatan Current on January 24, 1974. Air Expendable Bathythermographs (AXB) were dropped every 10 nm from a weather reconnaissance aircraft of the US Navy's VW-4 Squadron, Jacksonville, Florida. Profile A was flown first, beginning at 1828 GMT from $20^{\circ} 30' N$ $86^{\circ} 40' W$, the aircraft proceeding south-east for 190 nm. Skylab orbit overhead along the aircraft's track from 1831–1831:35 GMT. Profile B was started at 2138 GMT from $18^{\circ} N$ $83^{\circ} 40' W$, the aircraft proceeding north for 210 nm. The upward "doming" of cold water in the cyclonic eddies was encountered frequently along the first flight line, as expected in that the waters were well to the left of the current axis. (Note that the isotherm interval in the illustration is $1^{\circ} F$ to 70° ; then $5^{\circ} F$ to 60° . The vertical temperature gradient is nearly constant to depths of 1,000 feet.)

The temperature profiles (Fig. 3) indicated cyclonic eddies along the length of the flight line. The most dramatic modification of the ocean's thermal structure was in the vertical displacement of the deep-lying thermocline. Along the core of the current, the displacements of the isotherms were more subdued than to the west. It became clear from these data that the turbulent features were constantly forming and growing in the strongly flowing current.

The two most likely sources for the eddies are (1) sea-floor irregularities projecting into the surface layers, and (2) shear stresses along discontinuities in the current.

There are reefs on the Honduras Shelf over which the Yucatan Current flows before reaching the waters in which the eddies were photographed first. That the eddies show no organised shedding; such as Von Kármán or Kelvin-Helmholtz vortices, could simply be the result of multiple points of origin, thus masking organised structure. But, similar eddies and their uniquely associated clouds were observed from the Skylab 4 spacecraft in the Brazil Current (Fig. 2), the equatorial Pacific, the Coral Sea, the southern Caribbean Sea, and the Gulf Stream. In none of these areas do seafloor features jut into the surface currents. Such an origin does not seem to account, therefore, for the observed eddies.

The density discontinuity of the thermocline is well formed in the Yucatan Current⁴. A shear along this horizontal surface would result in turbulence, probably in the form of elongated "rols", however. A rotation around a near-vertical axis would be precluded⁵.

Probably, shear stresses across the velocity profile initiate the turbulence (as noted by Spilhaus¹, and calculated by Rossby) rather than the other causes considered. Wherever the eddies have been observed, the current velocity diminishes sharply over distances of only a few miles from that in the current core. That the eddy shape is not circular indicates that the velocity shear takes place along a sloping rather than a vertical plane which is consistent with velocity profiles in ocean currents.

Such turbulence has been studied in the laboratory⁶, and Brown and Roshko⁷ have examined it in detail and theorised on the development and growth of eddies as they move downstream. Should the Yucatan-type eddies be of this origin, then we must expect them to decrease in number and increase in size in a down-current direction; conserving vorticity through the sequence.

Some details from the ocean may come from an experiment planned for the Coral Sea in March 1974, by scientists in the Royal Australian, New Zealand and United States navies. The earliest opportunity to check the concept of down-current eddy amalgamation, which in the ocean must take place over distances of 1,000 miles, will be during the joint orbital manned mission of the United States and the USSR, in July of 1975. By mid-1975, a better definition of these unique ocean features may be possible.

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Lipid chemistry of eastern Mediterranean surface layers

It has been well established that organic surface films exist in most of the world's major oceans¹⁻⁹. They consist mainly of natural lipid material, with generally much smaller, though variable, amounts of petroleum hydrocarbons. Because the Mediterranean Sea is a restricted area the input of petroleum hydrocarbon is more noticeable than in most oceanic areas, and the level of surface pollution is probably increasing there because the major outflow occurs below the surface in the Straits of Gibraltar.

On a recent cruise of RRS Discovery in the Eastern Mediterranean, heavy oil slicks were encountered south of Cyprus and off the coasts of Libya and Egypt. Large coherent lumps of tar (tarballs) were often present with these heavy slicks, and in many places the associated surface films covered more than 60% of the surface area. In addition, when windspeeds were less than 10 knots, films not associated with oil slicks covered fairly large areas (30-40% at times). In certain coastal areas these surface films were accompanied by oil/water emulsions.

The surface films, emulsions, tarballs and surface living plankton were sampled at certain stations well away from heavy oil slicks in the eastern Mediterranean (Fig. 1). Their lipid composition and the extent to which petroleum hydrocarbons are entering the lipids of organisms which are exposed to high levels of oil pollution have been studied (Table 1).

A more detailed treatment of the results and sampling techniques will be published elsewhere¹⁰, but it should be emphasised that great care was taken to remove surface oil from organisms before analysis. The results can be summarised as follows:

Tarballs: in areas which were not grossly polluted by heavy slicks the distribution of tarballs was estimated to be 0.7-10 mg m⁻².

Emulsions: large scale distributions of oil/water emulsions were found off the Libyan and Egyptian coasts, possibly resulting from the practice of washing oil tanker's storage tanks down with high pressure water and discharging the washings before entering port. We made no estimate of the total organics present in the emulsion form, but a 54-mesh net collection gave a distribution of 0.1-0.5 mg m⁻² near the coast, with much lower concentrations in areas away from the coast. The emulsions were composed of both natural product organics (< 15% total extract) and a complex mixture of pollutant hydrocarbons (> 75% total extract).

Surface film: the surface film samples gave a distribution of 40-230 mg of organics per m², giving an estimated film thickness of 0.0005-0.003 cm. These values are similar to the estimate¹¹ that the oil slick observed six days after the stranding of the Torrey Canyon, which measured 10 by 40 miles, would have had an average thickness of 0.003 cm. The films were composed of both natural product lipids (< 5% total extract), which would be expected to form normal monomolecular films^{2,4}, and the complex mixture of pollutant hydrocarbons (> 85% total extract), which would give rise to polymolecular films^{9,12}.

Zooplankton: samples of near-surface crustaceans, fish and mixed plankton contained large amounts of hydrocarbons in their lipids (17-33% of total lipid; the lipid content of the animals was 2-3% of the total wet weight). These hydrocarbons

Table 1 Details of the samples

Sample number	Position	Type of sample	Estimated weight of tarballs (kg km ⁻²)	Estimated weight of emulsion (g km ⁻²)	Estimated weight of surface film (mg m ⁻²)	Hydrocarbon content of zooplankton (% of total lipid)
1*	35° 18' N 22° 5' E	Surface film	—	—	190	—
2	33° 8' N 22° 57' E	Tarballs Emulsion	6.1	400	—	—
3	32° 59' N 25° 52' E	Tarballs Emulsion	3.4	480	—	—
4	33° 11' N 27° 13' E	Tarballs Emulsion Crustaceans A Crustaceans B Fish	5.5 — — — —	350 — — — —	— — — — —	— — 30 19 17
5	33° 3' N 29° 4' E	Tarballs Emulsion	2.1	150	—	—
6	32° 35' N 30° 31' E	Tarballs Emulsion Crustaceans	0.7 — —	100 — —	— — —	— — 30
7	33° 27' N 31° 15' E	Tarballs Emulsion	10.0	30	—	—
8	35° 42' N 31° 41' E	Tarballs Emulsion	5.4	65	—	—
9	34° 37' N 31° 44' E	Tarballs Emulsion	1.9	110	—	—
10a	34° 26' N 33° 11' E (approximately)	Mixed zooplankton	—	—	—	33
b		Surface film	—	—	135	—
c		Surface film	—	—	200	—
d		Surface film	—	—	230	—
		Surface film	—	—	40	—

* Windspeed ≤ 10 knots.† Windspeed ≤ 4 knots.

were composed mainly of the complex mixture found in the emulsion and surface film samples, quite unlike the much simpler, natural product hydrocarbon composition normally found in unpolluted marine organisms¹³⁻²¹. In addition, natural product hydrocarbons are usually only minor components of zooplankton lipids ($< 5\%$ total lipid)^{22,23}.

These results confirm that the surface layers of the eastern Mediterranean are polluted with petroleum hydrocarbons in the form of surface films, slicks, oil/water emulsions and tarballs;

light penetration must all be considered among the long term physical effects of this pollution. The presence of high levels of non-natural product hydrocarbons in the lipids of the zooplankton samples is also a cause for concern. We suggest that either the zooplankton species examined do not have an effective metabolic mechanism for dealing with non-natural product hydrocarbons, or this mechanism has been overloaded by high levels of pollutants. It may well be that these zooplankton species can tolerate high levels of pollutant hydro-

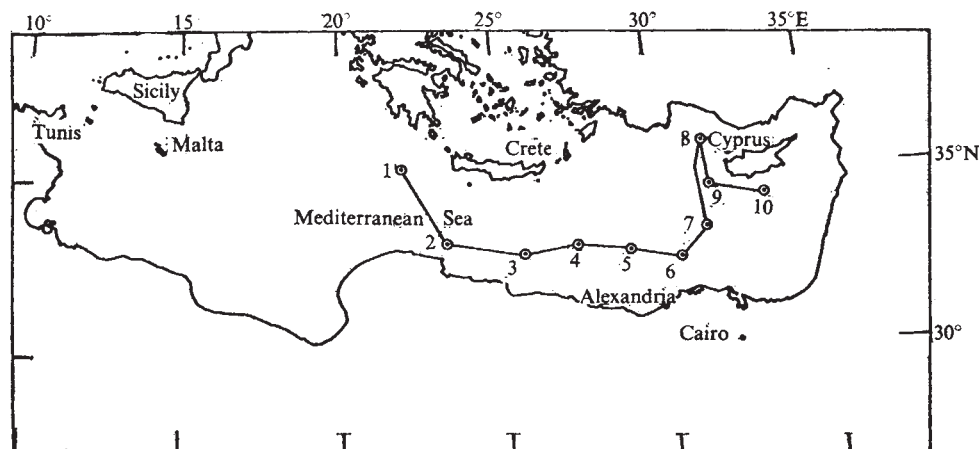


Fig. 1 Sample sites in the eastern Mediterranean. Numbers as in Table 1

in many places the natural monomolecular surface films are swamped by polymolecular oil films. The combined natural and non-natural film material covers large areas of the surface of this region under calm conditions. The influence on interface properties such as evaporation, solid/liquid exchange, gas exchange between the atmosphere and sea, wave formation and

carbons in their lipids without being adversely affected but, nevertheless, the near-surface plankton do represent a stage at which these compounds can be introduced into the marine food web.

In view of the increase in oil tanker traffic in the Mediterranean which will result from the re-opening and proposed

enlarging of the Suez Canal, it seems likely that the situation can only become worse.

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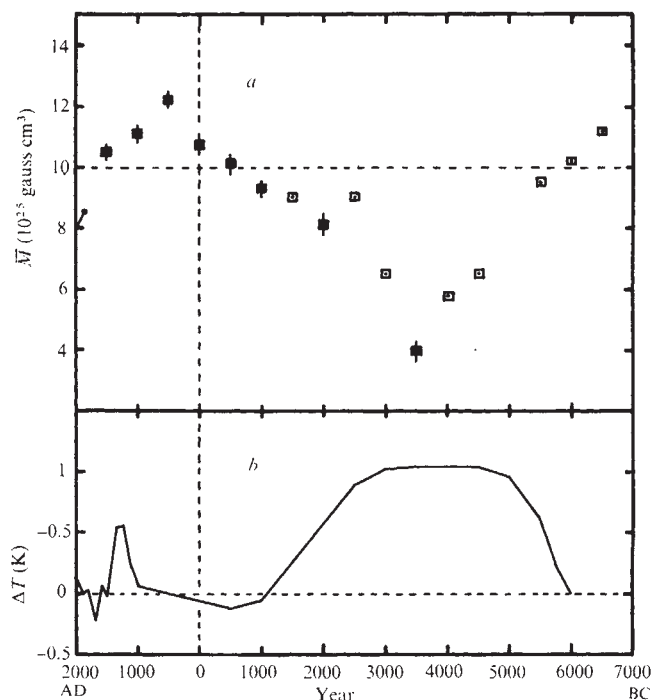


Fig. 1 *a*, World averaged dipole magnetic moment of the Earth for the past 9,000 yr (refs 5 and 6). Solid squares, averages with statistically significant number of samples. *b*, Damon's construction⁷ of deviations from the reference level (1890 AD) of long term averaged temperature. The construction is based on historical records and ocean cores.

half of its present value in the period 3000–5000 BC (Fig. 1*a*; refs 5 and 6), it may be expected that there was a major climatic response in the same period. Figure 1*b* shows a construction of the long term deviation in averaged temperature according to Damon⁷. Although the actual value of ΔT is debatable⁷, it is important to note that the major climatic optimum of postglacial times—3000–5000 BC—seems to stand in anticorrelation with the intensity of the geomagnetic field. As the climate may be affected by many factors, including changes in the solar constant and in the oceanic circulation this single correlation cannot be construed as evidence in support of King's¹ hypothesis.

The hypothesis that the westward drift of the semipermanent depression of tropospheric pressure follows the westward drift of the geomagnetic field pattern, can be tested by considering local archaeomagnetic and archaeoclimatic data according to longitude (Fig. 2). Similar variations in the archaeomagnetic field intensity—in units of the present value, B_0 —in both central Europe and Arizona–Mexico (Figs 2*a* and *c*) can, according to Bucha⁸, be interpreted as resulting from the westward drift of the northern geomagnetic field pattern (there are two regions of stronger field, over both regions): that would account for the 500-yr phase difference between the two sets of data.

The qualitative climatic variations of Greenland—midway between Central Europe and Arizona–Mexico—can be represented in terms of $^{18}\text{O}/^{16}\text{O}$ isotopic ratio deviations obtained from ice cores (Fig. 2*b*; ref. 9). In order to trace the possible westward drift of clear-cut geomagnetic and climatic events, I have identified on Fig. 2*a* some climatic episodes in central Europe. The occurrence of a cold period in Poland and Czechoslovakia between 800 BC, and 200 BC, is well established^{10,11}, although a later warm period may have been interrupted by minor cold episodes. The empirical association of this pre-Christian cold episode with the peak in geomagnetic field intensity at 500 BC, is compatible with the hypothesis that a semipermanent low may have been located in the north of central Europe at that time. The consistency of this empirical association, as well as the westward drift hypothesis, can be

Archaeomagnetism and archaeoclimatic 'forecast'?

It has been suggested¹ that the semipermanent pattern of depressions of tropospheric pressure in the north polar regions may be associated with areas of high magnetic field intensity in Canada and Siberia. The physical reasons for the correlation are obscure, because contrary to earlier suggestions, field lines near the magnetic pole are not linked to the interior of the magneto tail². Nevertheless, the hypothesis, as an empirical principle, is of wide interest in that it would not only organise archaeoclimatic data but it would also form a basis for long term forecasting of general circulation changes.

I here test some aspects of the hypothesis by correlating archaeomagnetic and archaeoclimatic observations from various regions of the globe. Although there may be a tenuous correlation between them in some cases, the total pattern of archaeoclimatic regimes from central Europe to western America does not seem to follow the westward drift of the geomagnetic field pattern. I suggest that climatic regimes may be composed of a global component—related to variations of the solar constant—and of a possible westward drifting component.

First, if there is geomagnetic control of the pressure pattern in the troposphere^{1,3}, then a climatic response to long term variations of geomagnetic field intensity may be expected over the whole globe. Indeed, if the main dipole field weakens, as at present, there is reason to believe that cosmic ray particles would be more readily accessible to the atmosphere⁴. Because the geomagnetic dipole moment, M , declined to approximately

tested. A cold episode occurred in Greenland at about 100–400 BC, some 200–300 yr after that in central Europe (Fig. 2b). (Although there are difficulties in the chronology of ice-core analysis, ref. 9 shows that the data are consistent with historical records in the short term, and with varve and ocean core chronology in the long term). On the other hand there is no consistent association between climatic and magnetic episodes in post-Christian central Europe between their westward drift to Greenland (Figs 2a and b). Nevertheless, if the sequence of climatic and geomagnetic associations during the cold episode in the first millennium BC is taken seriously, King's hypothesis would lead to the conclusion that such an episode would have drifted to western North America by about 200 AD, in accordance with the geomagnetic field drift (Fig. 2). The climatic record of the White Mountains of California has been derived from tree rings of the bristlecone pine (Fig. 2d; ref. 12). It can be seen that a cold episode did occur in the

first millenium AD; it was however, considerably later than 200 AD. Consequently, the archaeomagnetic and archaeoclimatic evidence available at present indicates only a tenuous correlation, at best.

In examining and interpreting the correlation of data discussed here, account must be taken of the preliminary nature of Bucha's⁸ interpretation of changes in field intensity observed for central Europe and western America. He suggested that they resulted from the westward drifting non-dipole field; even though the estimated rate of westward drift is in agreement with the better established nondipole drift of later epochs. As the data in Fig. 2 seem to be the only archaeoclimatic and archaeomagnetic data available at present for the same locations during the same epochs, I present them not only to indicate the present evidence for or against King's hypothesis, but also to emphasise the need for simultaneous and local data of both types. Evidence presented in support of the proposed hypothesis, including the original analysis¹, is based on isolated correlations; therefore, it seems that a systematic test, such as the determination of the westward drift of geomagnetic and climatic patterns, would be crucial in determining whether the hypothesis would be useful as an empirical principle for organising archaeoclimatic data. On the other hand, the organisation of climatic records according to longitude, such as in Figs 2a–d, does serve to distinguish global from local climatic episodes. Despite possible correlations with the geomagnetic field pattern, it is likely that the cold episodes at about 500 BC, and at about 1600 AD, are global in nature.

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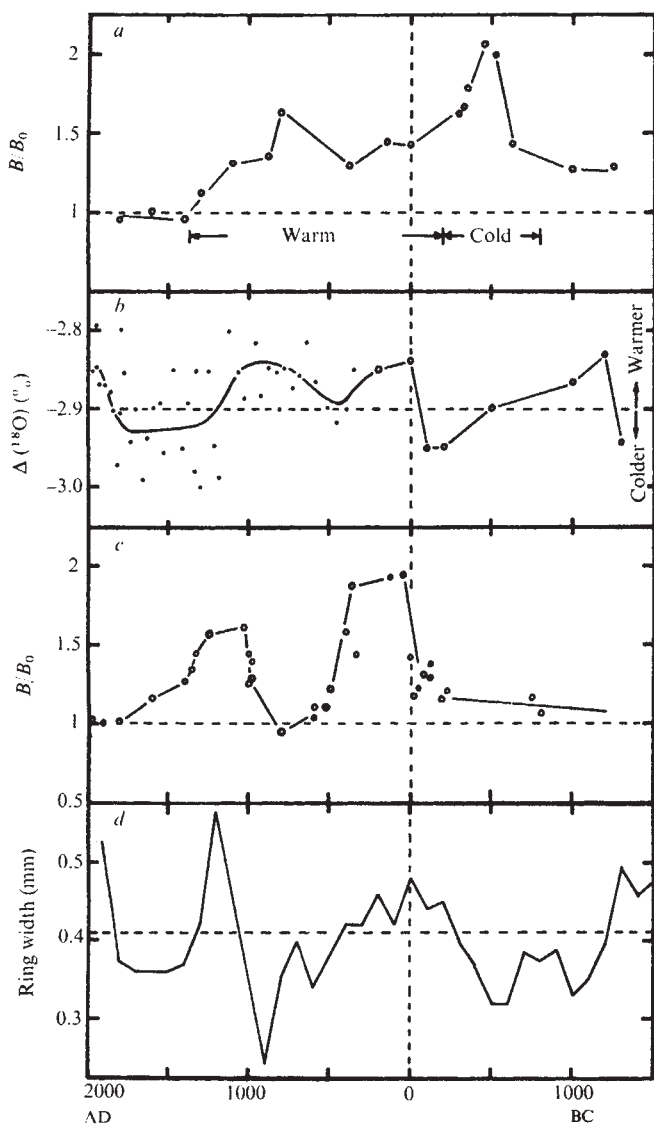


Fig. 2 a, Archaeomagnetic intensity, relative to the present value B_0 , for central Europe, 15° E (ref. 8). The indicated warm period, 200 BC–1400 AD may be interrupted by minor cold epochs. The Little Ice Age of circa 1600 AD is omitted. b, Archaeoclimatic data for Greenland (40° W) based on percentage deviation of $^{18}\text{O}/^{16}\text{O}$ isotopic ratio in ice cores⁹. Longer term averaged data before 200 AD taken from Fig. 4 of ref. 9. The continuous curve after 200 AD is drawn through the centroid of shorter term averaged data from Fig. 2 of ref. 9. c, Archaeomagnetic intensity, relative to the present value B_0 , for Arizona-Mexico, 105° W (ref. 8). d, Climatic record of the White Mountains of California (120° W) as indicated by the widths of bristlecone pine tree rings¹². In each case the horizontal dashed line is the mean.

Interaction of coherent electromagnetic waves with relativistic electrons in a medium

A MECHANISM is proposed here for producing coherent photons by the interaction in a medium of relativistic electrons (moving with velocity greater than the electromagnetic phase velocity in the medium) with coherent electromagnetic waves incident in the opposite direction. A new effect of potential use is exhibited; it is a consequence of two distinct physical phenomena acting synergistically to result in double-Doppler-shifted coherent waves contained within a Mach cone. Its applications include production of narrow band radiation at higher frequencies and wave numbers than are at present available from

lasers, which has implications for holography and for improved pumping efficiency and production of narrow band submillimetre waves from microwaves, which has significance for communications.

When a material body moves in a medium with a speed greater than that of the waves it produces in the medium, the energy accumulated in these waves cannot get ahead of the moving body, resulting in a wave with a sharp discontinuity on the Mach cone. In the field of hydrodynamics, the basic phenomenon is a shock wave. For a charged particle moving at a constant speed in a medium with index of refraction greater than unity, and with particle speed exceeding the phase velocity of light in the medium, the radiated wavelets emitted by the medium under the action of the field of the particle at different points of its path interfere constructively at the Mach angle, resulting in Čerenkov radiation. The radiation vanishes outside the Mach cone and peaks sharply near the edge of the cone.

A similar discontinuity and attendant enhancement of radiation near the edge of the Mach cone ought to occur because of the effect, on suitable media and at appropriate particle velocities, of the scattered-radiation field of a charged particle accelerated by a coherent electromagnetic field, but at the double-Doppler-shifted frequency that characterises Thomson (Compton) backscattering of laser light from energetic electrons.

Conversion of optical photons into ones of higher frequency in a vacuum by Compton backscattering of laser photons from energetic electrons has been observed^{1,2} in the laboratory. This vacuum effect^{1,2} does not fully exploit the coherent-wave nature of the laser source, and the original discussions³⁻⁵ of this effect did not consider the coherence properties of the incident radiation.

We extend these considerations by taking into account the coherence of the field driving the charged particle and by examining the laser-stimulated wave emission under conditions where the velocity of the scattered-field source exceeds that of field propagation in the medium. The properties of the scattered wave in these conditions are essentially different from Compton-backscattered waves in vacuum. We also discuss possible constructive interference from many-electron effects. The calculation is classical since the basic effect does not depend essentially on the quantum nature of light. Quantum corrections are small for most parametric ranges of interest.

We consider a relativistic electron moving with speed u , along the positive z axis, in an infinite medium of unit magnetic permeability. The electric field of the untruncated plane wave incident in the opposite direction (head-on collision) is

$$E_0(x, t) = E_0 \sin(\omega_0 t + k_0 z), \quad (1)$$

where $k_0 c = \omega_0 n$, c is the speed of light in vacuum and n is the index of refraction at the incident laser frequency. All quantities and results are in the rest frame of the dielectric. We consider first the case of a slightly dispersive medium, so that the dielectric constant ϵ at the scattered frequency can be reasonably approximated by a constant, though we include the possibility $\epsilon = n^2$, and take $\beta^2 \epsilon > 1$, where $\beta = u/c$. The scattered double-Doppler-shifted field (in cylindrical coordinates ρ, z) is, to order E_0 ,

$$E(\rho, z, t) = 2(r_e/\omega_0)(\gamma/\gamma_0)E_0 \frac{\partial}{\partial t} \left\{ (\tau) \Theta(\gamma u \tau - \theta) \right. \\ \left. \times \cos[\Omega(\gamma^2 \tau - z/u)] \frac{\cos[\alpha(\gamma^2 u^2 \tau^2 - \rho^2)^{1/2}]}{(\gamma^2 u^2 \tau^2 - \rho^2)^{1/2}} \right\} \quad (2)$$

where r_e is the classical electron radius, $\gamma_0 = (1 - \beta^2)^{-1/2}$, $\gamma = (\beta^2 \epsilon - 1)^{-1/2}$, $\theta(x) = 1$ for $x > 0$ and $\theta(x) = 0$ for $x < 0$, $\tau = t - (z/u)$, $\Omega = \omega_0 + k_0 u$, and $\alpha = \gamma \Omega \epsilon$.

The component K_z of the wave number for propagation of the scattered wave in the z direction is

$$K_z = (\Omega_z/u)(1 + \gamma^{-2}) \quad (3a)$$

where

$$\Omega_z = \gamma^2 \Omega = [\omega_0(1 + n\beta)/(\beta^2 \epsilon - 1)] \quad (3b)$$

is the corresponding frequency of propagation; that is, this component of the wave has a phase velocity v_z dictated in part

by the electron velocity,

$$v_z = u(1 + \gamma^{-2})^{-1} = c/(\beta \epsilon) \quad (4)$$

Note that the maximum enhancement of the frequency, at least for the simple model considered here, occurs just above threshold for the effect. As β increases beyond threshold, for a constant ϵ , Ω_z decreases. This differs qualitatively from the vacuum effect, where the scattered frequency increases monotonically with β . The effect can therefore be optimised for certain parametric ranges by suitable adjustment of ϵ for a given medium, for example, by varying the density of a gas. Scattered coherent waves of 10^2 to 10^3 times incident infrared frequencies should be realisable in low-density gases with $0 < \epsilon(\Omega_z) - 1 \ll 1$.

The scattered field vanishes outside the Mach cone of half angle $\phi = \sin^{-1}(v_p/u)$, where $v_p = (c/\epsilon)^{1/2}$ is the phase velocity of electromagnetic disturbances in the medium; $v_z = v_p \sin \phi$. The intensity increases sharply as the conical edge is approached. The singularity in Equation (2) for the field on the cone is in reality smoothed out by the absorptive properties of a dispersive medium, as in the Čerenkov case, and by energy losses of the charged particle, which serve to place a lower limit on the parameter $Q = \gamma^2 u^2 \tau^2 - \rho^2$ that results in the singularity for $Q = 0$ when dispersion and these losses are not taken into account.

To estimate the field energy of the scattered double-Doppler-shifted wave, we keep only the dominant contribution to the expression (2) in the frequency range 10^{14} to 10^{18} rad s⁻¹ for ω_0 and take the energy loss in the medium into account phenomenologically. The ratio η of the energy scattered from a single electron to that of the portion of length L of the laser beam which overlaps the electron beam in the dielectric is then $\eta = (2\pi r_e^2/A)(L/L_i)(\gamma/\gamma_0)^2(\Omega_z/\omega_0)^2(\beta^2 \epsilon)^2(\Omega_z \tau_a)^2 \ln(R/2L_i)$, (5)

where R is the range of the electrons, L_i is the distance over which the Mach cone builds up (the interaction length), A is the cross section of the laser beam and τ_a is a representative value of τ during the time T of interaction of a single electron with the incident wave in the medium. We assume the total lengths of the laser and electron beams to be larger than the dielectric thickness, which is readily achievable and renders independent of these lengths the ratio η and, for fixed electron beam density, the quantities η_{coh} and η_{incoh} given below. By definition, we then have $L \simeq L_i$, and geometry of the cone dictates $\tau_a \simeq T/3$. From equations (5) and (3b), η is proportional to γ^{10} , so that a change of $\beta^2 \epsilon$ from 1.2 to 1.05, for example, increases η by three orders of magnitude. We have, however, made no systematic effort to optimise the various relevant parameters such as dielectric constant, electron energy, or pulse time and total energy of either the electron or laser beam.

Expressions (2) and (5) apply to scattering from a single electron. The number N of electrons in the beam that act at a fixed time to produce the desired effect is given in terms of the total number N_0 of beam electrons by $N = N_0(d/L_e)$, where we assume the electrons to be uniformly distributed in a beam of length L_e and d is the thickness of the dielectric. Two limiting cases will be considered.

For electrons bunched in space-time so that the resulting phases $(\Omega_z t - K_z z)$ of the waves scattered from different electrons are all within, say, the same quadrant, these waves will add constructively. Because the electron and wave velocities are nearly equal, waves scattered from electrons bunched within $1/4$ wavelength λ of the scattered radiation will also be within $1/4$ period of one another. Such constructive interference from randomly distributed electrons may be realisable by using a thin dielectric film of thickness $\lesssim \lambda/4$. The number N_e of electrons in the beam that can act coherently is then given by $N_e = N_0(\pi c/2\Omega_z L_e)$. Additional enhancement may be obtainable by using a suitable array of periodically spaced thin films, by modulating the electron beam and by time phasing of the laser pulse. In adding coherently the contributions from different electrons, one should in principle fold in a factor for the spread in electron velocities. In practice, the phenomeno-

logical treatment referred to above takes this partly into account. For an interaction region larger than a wavelength, the total intensity of radiation from randomly distributed electrons will be the incoherent sum of the individual electron contributions. The ratio for coherent scattering from a beam of electrons interacting in a single thin dielectric, $d \approx \lambda/4$, is $\eta_{\text{coh}} = N^2 \epsilon \eta$. For $d \gg \lambda/4$, we get $\eta_{\text{incoh}} = N \eta$.

As a specific example, we consider an electron beam that delivers $N_0 = 10^{18}$ electrons in a 5×10^{-8} s pulse at an average energy of 5 MeV ($L_e \approx 1,500$ cm, $\gamma_0 \approx 10$), and a dielectric of thickness $d = 1$ mm, $R = 1$ cm and $\epsilon = 1.2$, so that $\gamma^2 \approx 5$ and $\Omega_e/\omega_0 \approx 10$. Taking an infrared laser (10^{14} Hz), for which $\Omega_e \tau_a \approx 6,000$ produces an ultraviolet incoherent scattered wave of relative intensity $\eta_{\text{incoh}} \approx 10^{-2}$. For a thin film of $d = \lambda/4 = 7.5 \times 10^{-6}$ cm, the coherent scattered wave is in the ultraviolet with $\eta_{\text{coh}} \approx 4 \times 10^{-5}$.

The portion of the electron beam that can contribute to the process will in general be limited by the time over which the essential dielectric and material properties of the medium are not destroyed by the laser or electron flux. Estimates for glass indicate no significant change in the electric susceptibility for the above laser and electron parameters. But for a 5-MeV electron beam with $N_0 > 10^{15}$, the physical state of the glass may alter before all the electrons have passed through.

The effect can also be used to generate coherent millimetre and submillimetre waves from intense coherent microwaves, such as from magnetrons, in slow wave or periodic structures (simulated dielectric media). In the case of such simulated dielectric media, where the effect we consider can actually take place in vacuum, the duration and intensity of the scattered radiation are not limited by either the dielectric or the material properties of a medium.

The discussion up to now has involved scattered radiation at energies below those at which atomic resonances occur, for which the assumption of slowly varying electric susceptibility is expected to be valid. The general nature of the physical mechanism underlying the effect we have discussed strongly suggests however that the qualitative features of the effect will persist in the region of anomalous dispersion corresponding to X-ray and γ -ray frequencies, inasmuch as the electric susceptibility for suitable media can be positive in these frequency ranges^{6,7}.

By adjusting such radiation to resonance with the energy required to excite some metastable state, the effect can be applied to achieve substantial population inversion at these higher frequencies. Such selective pumping to the metastable state would improve pumping efficiency over either flash or electron-beam excitation alone. The medium in which the radiation is produced can be, but does not have to be, the same as the system being pumped.

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Intermolecular basis of odour

THREE quite different theories of odour discrimination are in favour at present. The 'site filling theory' of Amoore¹ relates molecular shapes to odour types and identifies some classifications as primary. The penetration and puncture theory of Davies² develops a physical model for impulse transmission. The key physical properties involved are the molecular size and the adsorption coefficient between air and a lipid-water interface. Wright³ has correlated vibrational frequencies in the far infrared with the quality of odour.

All these theories, however, do agree that although there is no clear relationship between the functional group(s) present and odour, the mechanism of interaction with the receptor(s) must be physicochemical involving either electronic, vibrational or rotational forces or any combination of these.

Here I present evidence which implicitly correlates both odour quality and odour thresholds with the depth of the intermolecular potential energy well formed between the odour-producing molecule and the unknown receptor site.

There is a relationship between the depth of the pairwise intermolecular potential energy well and the normal boiling point of a substance⁴; the depth of the well increases linearly with the boiling point (K). The negative derivative of this potential with respect to intermolecular separation is a measure of the electronic interactive force.

In the case of the interaction of a molecule with the receptor site the depth of the potential well formed will depend on the nature of the charge distribution of the odorant molecules. If only one basic type of receptor site is assured, with numerous formal charges, then some correlation should be possible between the normal boiling point of a molecule and its odour type. I have found the normal boiling points for 253 of 418 ethereal, camphorous, minty, almond, aromatic, floral and musk compounds listed by Amoore¹. Table 1 summarises the results giving average

Table 1 Average boiling points and standard deviations of different odour classes

Class*	Average boiling point (K)	s.d. (K)
Ethereal	346	66
Camphorous	450	40
Minty	453	36
Almond	457	45
Aromatic	482	49
Floral	498	48
Musk	636	81

* Pungent and putrid classes were not considered since these can be understood in terms of highly reactive functional groups.

values and standard deviations for each odour class. Ethereal compounds have the lowest boiling points with the musks at the other extreme. Both of these have average values quite far removed from those of the other groups (camphorous, minty and almond) whose average values are so close to one another as to be indistinguishable. This is interesting in that many of the compounds possess more than one of these three odours at a time. The lowest boiling compound of all the molecules is ethylene (ethereal, 169 K). Very low boiling compounds such as CO (81 K), CH₄ (112 K), O₂ (90 K), N₂ (77 K) have small interactive electronic forces with the receptor site and hence are odourless.

A better correlation is achievable if boiling points of compounds with the same functional group are classified separately. Table 2 lists saturated halides in terms of increasing boiling points. If one assumes that for the halides 377 K is the cutoff for ethereal compounds then one obtains

Table 2 Boiling points (b.p.) and odour classifications (o.c.) of alkyl halides

Alkyl halides	b.p.	o.c.*	Alkyl halides	b.p.	o.c.
Methyl chloride	249	E	Pentachloroethane	435	E
Methyl bromide	278	E	1,3-Dibromo 2, 2-dimethyl propane	460	E
Ethyl chloride	285	E	1,1,2-Tribromo 1,2,2-trifluoroethane	390	C
Ethyl bromide	311	E	3-Chloro 2,2,3-trimethylbutane	403	C
Methylene chloride	313	E	Hexachloroethane	458	C
Methyl iodide	318	E	1,1,2,2-Tetrabromo-chloroethane	475	C
Propyl chloride	329	E	1,1,2,2-Tetrabromo-difluoroethane	477	C
Chloroform	334	E	1,1,2-Tribromo-cyclobutane	503	C
Ethyl iodide	345	E	1,2,2,3-Tetra-bromopropane	528	C
Carbon tetra-chloride	350	E			
1,2-Dibromo 1,1,2-trifluoroethane	350	E			
Propyl iodide	375	E			
1,2,2,2-Tetra-bromoethane	377	E			
Bromoform	422	E			

Boiling point at 1 atm (K). Some of the values have been extrapolated from reduced pressure boiling points. With some of the musks we can only give an inequality but these are reasonable.

* Odour classification code. E, ethereal; C, camphorous.

$\lambda^2 = 0.25$ and $P = 0.38$. Similar success is obtained when looking at other functional groups.

In Table 3 the negative log of the odour threshold concentration is computed for a series of saturated alkanes and compared with experimental values³. The thresholds (negative log of the concentration) are computed by assuming the correct value for ethane and that the other thresholds should vary by the proportion of their boiling point (K) to that of ethane (K). These results give a high correlation $\lambda^2 = 1.22$ and $P = 0.005$. Since the potential well depth increases with increasing boiling point, fewer molecules would be needed to trigger an impulse. Similar success is obtainable with other classes of molecules.

Table 3 Computed compound with experimental values of the negative log of the threshold concentration for a series of alkanes

Compound	T (K)	Calculated threshold*	Experimental threshold†
Ethane	185	—	3.08
Propane	231	3.85	3.31
Butane	273	4.55	4.28
Pentane	309	5.14	5.32
Hexane	342	5.69	5.57
Heptane	371	6.18	5.79
Octane	399	6.64	6.21
Nonane	423	7.04	6.33
Decane	446	7.43	7.10
Undecane	468	7.79	6.83
Dodecane	488	8.12	6.66
Tridecane	507	8.44	6.64

* Negative log of the threshold concentration (mol l^{-1}) calculated by taking $(T_b(\text{K system})/(T_b(\text{K}) \text{ ethane}) \times (\text{negative log of ethane threshold}))$. (T_b = normal boiling point).

† Negative log of experimental threshold concentration (mol l^{-1}) determined from values in ref. 5.

The theory outlined above fits in with the Davies theory, since it may be the mechanism by which the odorant molecule opens up the space in the cell membrane to induce charge flow. The theory above also gives support to Wright's vibrational correlations since the vibrational energy level separations reflect the shape of the potential energy curve and hence the rate of energy transfer. It appears that the difference between minty, camphorous, and almond odours has more to do with the shape of the potential energy curve than with the depth of the well. In these terms the nearly infinite nuances of odour can be understood.

Randebrock⁶ has suggested that the α -helix protein

molecule may be a receptor site for odour. It should now be possible using electrostatic models based on quantum mechanical charge densities for such molecules to see if a correlation of potential energy surface and odour can be established for this or any other suspected receptor molecule.

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Coevolution of Danaid butterflies with their host plants

MALE butterflies of the nymphalid subfamily Danainae possess pheromone disseminating organs (hairpencils) the secretions of which function as female flight arrestants or aphrodisiacs^{1,2} and in some case as female attractants¹. The hairpencil secretions of a number of Danaid species contain dihydropyrrolizine derivatives³⁻⁸ which are obtained from pyrrolizidine alkaloid plants by adult feeding^{6,7}. A dihydropyrrolizine ketone isolated⁸ from the hairpencils of *Danaus gilippus herenice* Cramer has been shown to be the flight arrestant or aphrodisiac pheromone in this species^{2,9} and we believe that the related dihydropyrrolizines found on the hairpencils of other species may perform a similar function. We propose here one possible explanation for the unusual dependence of male Danaid butterflies on pyrrolizidine alkaloid plants.

Many Danaid species apparently store in their body tissues vertebrate heart poisons (cardenolides) sequestered by their larvae from food plants^{10,11} which are found primarily in the cardenolide containing sections of the Asclepiadaceae and Apocynaceae¹². The Danaiids' association with cardenolide plants is considered to be an important factor protecting them from vertebrate predators.

The pyrrolizidine alkaloid plants most commonly sought out by adult males are species of the Boraginaceae which contain the 1,2-unsaturated allylic ester type alkaloids¹³ for which metabolism into the hairpencil dihydropyrrolizines is readily envisaged. Other sources of this particular type of pyrrolizidine alkaloid which are visited by male Danaiids, are the tribes Senecioneae and Eupatorieae of the Compositae and the genus *Crotalaria*¹⁴ of the Leguminosae¹³.

We suggest that the Danaid's use of, and dependence on, pyrrolizidine alkaloids may have developed during a period when these alkaloids were constituents of their larval food plants. Based on current views of the phylogeny of the plants concerned we propose that the early larval food plants contained both pyrrolizidine alkaloids and cardenolides and that they, perhaps under pressure of insect predation (see below), split into a cardenolide and a pyrrolizidine alkaloid branch. The Danainae then retained protection from predators by evolving as larval feeders on the cardenolide plants with the males visiting pyrrolizidine alkaloid plants to obtain their pheromones.

Our proposal is consistent with the phylogenetic classifications of Cronquist¹⁵ and Takhtajan¹⁶ if the ancestral food plants occurred early in or before the evolution of the subclass Asteridae and diversified according to the scheme shown in

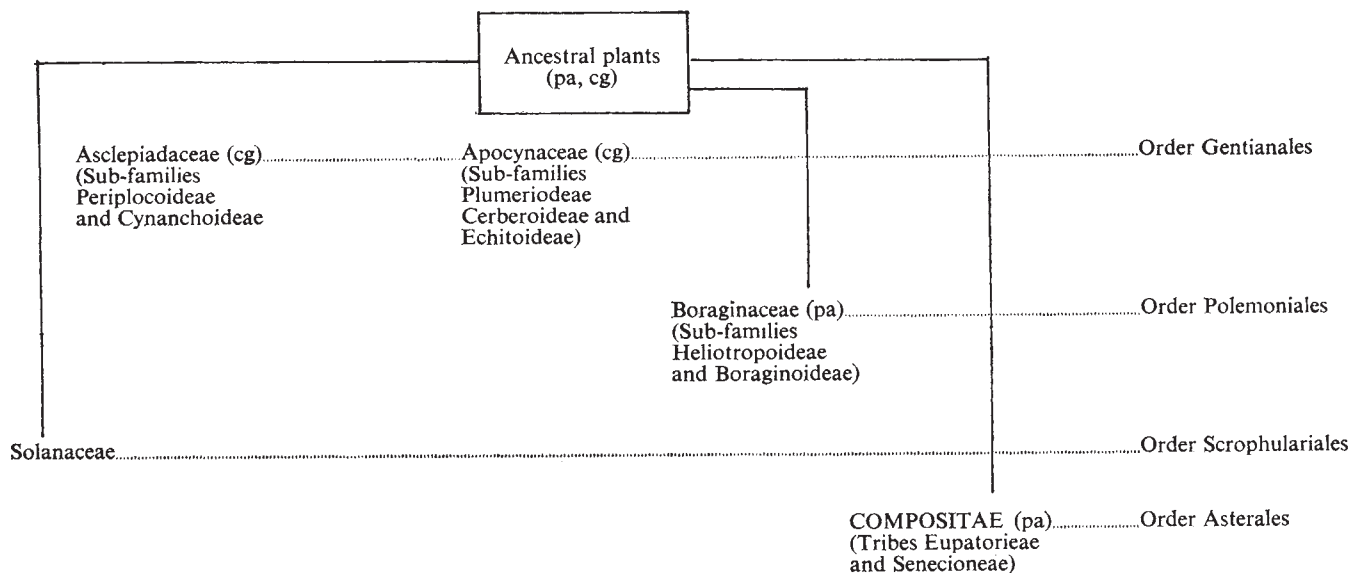


Fig. 1 Proposed origin of the pyrrolizidine alkaloid- and cardiac glycoside-containing sections of the plant families in the subclass Asteridae with which Danaid and Ithomiid butterflies associate showing the distribution of cardiac glycosides (cg) and pyrrolizidine alkaloids (pa).

Fig. 1. Of the pyrrolizidine alkaloid plants which male Danaids visit, only *Crotalaria* species are found outside this subclass. They may be an earlier branch of the postulated ancestral host plants (compare with Takhtajan¹⁶) or represent an independent development of this particular type of pyrrolizidine alkaloid.

Divergence of the original food plants might have occurred as a result of the dependence of the early Danaids on pyrrolizidine alkaloids and cardenolides. Because of their importance to the Danainae both of these substances, or related marker substances, probably provided the cues for locating, and the stimulus for ovipositing on, the early larval food plants. This would have favoured the survival of plants which were naturally deficient in one or other of these chemicals.

The possibility of co-occurrence of cardenolides and pyrrolizidine alkaloids in progenitor species is exemplified by *Urechites karwinsky*, Mueller, (Apocynaceae) (also referred to as *Fernaldia pandurata* (A.D.C.) Woodson¹⁷). A dihydropyrrolizine closely related to the Danaid hairpencil dihydropyrrolizines has been isolated from this toxic plant¹⁸ and other toxic species of the genus are known to contain cardenolides^{19,20}. We regard as highly significant the fact that *U. karwinsky* is used as a larval food plant by a primitive Ithomiid butterfly, *Tithorea harmonia salvadores* Staudinger, whose larvae bear a close affinity to those of certain Danainae²¹. The subfamily Ithomiinae (Nymphalidae) is closely related to the Danainae²¹ and in common with male Danaids, male Ithomiids are attracted to, and feed on *Heliotropium indicum* L. (Boraginaceae), which contains pyrrolizidine²². Their larvae feed primarily on Solanaceae¹² but notable exceptions are species of the genus *Tithorea* whose

larvae feed, like certain of the Danainae, on Apocynaceae²¹. The *Tithorea harmonia salvadores-Urechites karwinsky* association may therefore be a conservative development from, and provide a model for, the ancestral association we have proposed.

By considering the pyrrolizidine plants visited by adult males to have evolved from earlier larval food plants the present behaviour of the Danaids and the development of the hairpencil chemicals as female flight arrestants and attractants can be more readily understood. Thus, it is envisaged that mating of early Danaids occurred in the vicinity of the larval food plants and that males subsequently developed the means of storing and disseminating volatile pyrrolizidine alkaloid-derived plant substances then used by the females to locate the larval food plants. By disseminating these substances in the vicinity of a female in flight they would be able to make her settle and thus effect mating in a place remote from the larval food plant. Such a development would have offered an expanded habitat for the insects and would have become essential for their survival during divergence of the original food plants when the females were being selected for their ability to find and oviposit on cardenolide plants, which gave their offspring protection from predators, while the males were seeking out pyrrolizidine plants.

Males make up over 90% of the butterflies attracted to pyrrolizidine plants but a small proportion of females of some species also visit them^{6,23} (Table 1). This may be the remnant of a behaviour pattern and olfactory acuity associated with location of the earlier larval food plants which has not yet been completely lost from the gene pool of the females. *Tithorea* species again seem to be exceptional and, in agreement with the

Table 1 Typical collections of Ithomiinae attracted to *Heliotropium indicum* L.

Species	Males	Females
* <i>Ithomia iphianassa iphianassa</i> D.-H.	166 (98.2%)	3 (1.8%)
* <i>Hymenitis darcetis</i> H.-S.	294 (99.7%)	1 (0.3%)
* <i>Pteronymia beebei</i> Fox and Fox	329 (94%)	21 (6.0%)
† <i>Tithorea harmonia megara</i> Latr.	3 (60%)	2 (40%)
‡ <i>Tithorea terracina pinthias</i> Godman and Salvin	2 (20%)	8 (80%)

* Collected at Estacion Rancho Grande, Parque Nacional Henri Pittier, Aragua, Venezuela; July–August; 1972–73.

† Collected at Wm. Beebe Research Station, Arima, Trinidad; July 1962.

‡ Collected at Cerro Campana, Panama; April 1971.

conservative development we have proposed for Tithorea, both males and females are attracted (Table 1).

The existence in the past of plants containing pyrrolizidine alkaloids and cardenolides might explain Rothschild's observation²⁴ that certain moths of the families Arctiidae and Ctenuchidae also utilise both these phytochemicals.

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'Fimbriae' in the fungus *Ustilago violacea*

LONG fine hairs, called fimbriae¹, or pili², are commonly found attached to the cell walls of Gram-negative bacteria³, but do not seem to have been reported in other organisms. We report here that the yeast-like sporidial cells of the anther smut fungus *Ustilago violacea* also produce fimbriae. At present the use of the term fimbriae for these fungal hairs merely denotes a close morphological and developmental similarity to the bacterial structures; however, preliminary investigations of the fungal 'fimbriae' indicate that there may be chemical and functional similarities too.

The fimbriae of *U. violacea* can readily be observed when sporidia from a log-phase population are washed at least three times in particle-free distilled water, and then air dried on a Formvar-coated grid before shadowing with

tungsten oxide at an angle of 18–25°. The fimbriae resemble bacterial fimbriae in the following ways.

(1) They are of similar dimensions, being about 1–10 µm long and 60–70° A in diameter. They are curved and flexible and do not branch or taper. The apparent branching of the fimbriae in Fig. 1 is common and occurs when they twine together to form multistranded 'cables' which may dissociate into the individual strands. They resemble the curved fimbriae of *Vibrio* spp.³ rather than the rigid, straight type I characteristic of *Escherichia coli*² and *Salmonella*¹. We cannot be certain whether or not they have the hollow helical structure characteristic of these type I and F fimbriae, but apparently absent from the *Vibrio* forms.

(2) Fimbriae originate below the cell wall and penetrate through it. Freeze-etched preparations show particles of about 70–100 Å on the outer surface of the plasma membrane and corresponding projections on the inner surface of the cell wall. Sectioned walls are traversed by fibrils 70–100 Å in diameter which apparently are fimbriae passing through the cell wall.

(3) As in bacteria, populations of cells in logarithmic growth are the most fimbriated, while stationary phase cells are hairless. Broth cultures are much more fimbriated than cultures from nutrient agar plates. But, even in heavily fimbriated populations, the quantity of fimbriae on individual cells appears to vary greatly from 0 to more than 200. Similar variation has been found in bacteria². The fimbriae seem to be susceptible to mechanical damage and may contract into tight coils on the surface of most cells in particular regions of a grid. Similar coils are produced on all cells by treatments with various reagents including the fixatives glutaraldehyde and formalin.

(4) The fimbriae can be removed easily from cells by violent mechanical agitation in a blender, by sonication or by centrifugation through a viscous liquid (for example, 40% sucrose). In bacteria, fimbriae regenerate very rapidly, beginning about 10 min after reinoculation into nutrient medium². Similar rapid regeneration occurs in *U. violacea* as the fimbriae begin to appear in less than 1 h and reach normal length (about 3–7 µm) in less than the cell cycle time of 5 h. Again as in bacteria the fimbriae are constantly produced and shed by cells so that many free unattached fimbriae can be observed in all cultures.

(5) Type I bacterial fimbriae are composed mainly of a protein called pilin². The fimbriae of *U. violacea* are completely digested by treatment with pronase and thus contain protein throughout their length. Treatments with ether, DNase or RNase do not affect them, and they are not stained by phosphotungstic acid (PTA) or ruthenium red. We conclude that they may be largely and perhaps entirely composed of protein.

We are examining other yeasts and yeast-like fungi to determine whether such fimbriae are common. No species we have investigated so far reveals the long fimbriae of *U. violacea* and indeed even the strains of this species maintained by the Centraalbureau voor Schimmelfcultures, Baarn, The Netherlands, show fewer fimbriae. However, short hairs and other extensions are produced by some yeasts in genera such as *Lipomyces*, *Nadsonia* and *Saccharomyces*. Thus the presence and structure of surface hairs may have significance for the taxonomy of yeast-like fungi. We have observed short hairs in *Saccharomyces cerevisiae* which are probably extracellular extensions of the microfibrils that Moor and Mühlethaler observed arising from particles on the plasma membrane and penetrating into the wall⁴. These hairs are more frequent on flocculent strains than on non-flocculent forms.

What role do the fimbriae play in the growth of the smut cell? Even though bacterial fimbriae were described about 20 yr ago the functions of the several kinds are largely unknown, except for the F fimbriae which are thought

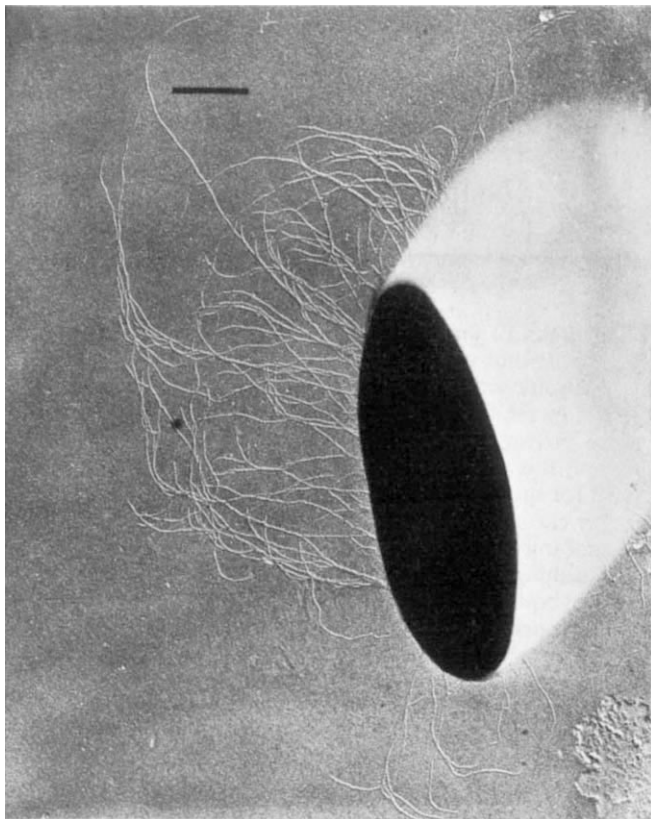


Fig. 1 Metal shadowed preparation of a sporidium of *Ustilago violacea*, showing many long flexible fimbriae. The apparent branchings are always associated with reduction in width and are due to dissociation of multistranded 'cables'. The bar represents 1 μ m.

to transport DNA molecules from F⁺ or Hfr cells to F⁻ recipients. Brinton² has suggested that the other fimbriae are involved in transport of metabolites or informational macromolecules, colicin-like killing of other cells, or simply adhesion to surfaces. He has also suggested that some fimbriae are rod shaped viruses being released from the cell in a manner similar to the release of the DNA male phages.

The function of fungal fimbriae is at present a matter of speculation. The suggestions made above for bacteria are equally applicable to the yeast-like *Ustilago* cells. One possible role would be in the mechanism of conjugation. We have shown that development of the conjugation bridge depends on mutual information transfer at an early stage in courtship^{5,6}. We speculated that such information transfer was mediated 'probably through hairs on the glycocalyx'. The fimbriae described here provide a firm morphological basis by which this information transfer could occur, as we have evidence showing that fimbrial connections are formed between the cells in the early stages of the conjugation process. We are investigating the synthesis and structure of the fimbriae in *U. violacea* and their possible role in conjugation. A full account of this work together with a description of fimbriae in other yeast-like species will be published elsewhere.

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***In vivo* hybridisation of human tumour and normal hamster cells**

It is exceedingly rare for neoplasms to be invasive and metastatic when grafted in xenogeneic hosts¹. During the past 7 yr, we have on numerous occasions observed the production of invasive and metastatic tumours in hamsters soon after injecting human cancer cells of diverse histopathology into their cheek pouches²⁻⁷. We have interpreted this biological phenomenon as due to the *in vivo* fusion of the human tumour with normal hamster host cells²⁻⁸, although the involvement of oncogenic viruses was not excluded. Recently, other evidence supporting the hybridisation of tumour with normal cells *in vivo* has appeared⁹. The significance of such events in the neoplastic process, however, has yet to be appreciated.

It is our view that such *in vivo* cell hybridisations in xenogeneic and perhaps also in isogenic systems are a means by which neoplastic cells can progress to more advanced states of malignancy, as exhibited by the properties of invasiveness and metastasis^{8,10}. Utilising advances made in methods for individual chromosome identification, we have now obtained karyological evidence suggesting the *in vivo* fusion of transplanted human cancer cells with putatively normal hamster cells, resulting in the formation of very lethal neoplasms.

As performed in previous studies²⁻⁷, a portion of an astrocytic glioma (Fig. 1a) from the brain of a 44-yr-old female was injected as a fine cell suspension into the cheek pouches of nine male golden hamsters (*Mesocricetus auratus*) weighing 50-60 g. No immunosuppressive host-conditioning measures were used. Fourteen days later, a large tumour measuring 1.1 cm in diameter was confirmed in only one animal's cheek pouch. The microscopic appearance of the tumour graft did not resemble that of the original human neoplasm (Fig. 1b). Four weeks after transplantation, this animal expired with widespread metastasis to all major organs, including the lungs (Fig. 1c) and the adrenals (Fig. 1d). Aliquots of the first passage cheek pouch tumour were then grafted to other hamsters, in their cheek pouches or intraperitoneally, as well as explanted in cell culture. Our cell culture techniques have been described elsewhere¹¹. Both *in vivo* and *in vitro*, a continuously propagable tumour cell line resulted, and has been termed GB-749. Either growing in the cheek pouch or in the ascites form, GB-749 has been uniformly lethal for its hamster host.

GB-749 cells from the fifth ascites and fifteenth cheek pouch tumour passages were placed in culture for 6 and 18 d, respectively, before collecting for cytogenetic analysis; the cancer patient's peripheral blood cells were treated similarly, except for the addition of phytohaemagglutinin, according to standard cytogenetic methods¹². Counting the chromosomes of 50 GB-749 cells of each passage revealed that between 52 and 60% of the cells had a modal range of 58-60 chromosomes. Twenty cells of each transplant generation were karyotyped after banding the chromosomes by the trypsin-Giemsa method¹³, and all of the aneuploid tumour cells examined contained both hamster- and human-like chromosomes. The karyotype of a representative GB-749 cell is reproduced in Fig. 2a, showing chromosomes with banding patterns typical for both human and hamster chromosomes.

Table 1 Frequency of human and hamster chromosomes (single or pairs) in 20 karyograms of GB-749 hybrid tumour cells

		Fifth ascites passage																				
Chromosome No.	X	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Hamster	18	17	12	4	11	20	20	19	14	13	20	19	20	16	19	19	20	17	19	19	15	2
Human	0	2	13	4	0	1	1	2	0	1	0	7	3	0	0	0	0	0	10	0	0	14

		Fifteenth cheek pouch passage																				
Chromosome No.	X	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Hamster	10	10	17	5	13	19	20	18	16	19	18	19	20	18	20	20	20	17	18	20	20	4
Human	0	0	10	8	0	0	0	1	0	5	1	1	0	1	0	1	1	0	7	0	0	15

Table 1 shows the frequency of each hamster and human chromosome identified in the fifth and fifteenth hamster transplant passages of GB-749. Chromosomes with a hamster-like banding pattern predominated in both cell generations. A significant number of the human-appearing chromosomes, however, No. 2, 3, 11, 18, and 21, were present among the 20 karyotypes examined at random.

The resemblance of the banding patterns of the human-like chromosomes most frequently found in GB-749 to their counterparts in the patient's peripheral lymphocytes is demonstrated in Fig. 2*b*. At times, the hamster's chromosome No. 9 looked very similar to the human chromosome No. 11 and 12, but the ones chosen in this figure clearly indicate the presence

of the human 12 chromosome in GB-749. Of additional interest is the occurrence of pronounced satellites on the short arms of the 21 chromosome, both in the patient's peripheral blood cells (H) and in the GB-749 tumour (T) produced in hamsters and serially propagated in these animals and in cell culture. In addition, this chromosome had a centromeric banding pattern typical for the human 21 and distinct from that of any normal hamster chromosome of this size and form. Our recent identification of small acrocentric chromosomes, however, with similar distinguishing features in mouse melanoma/hamster cheek pouch hybrids produced *in vitro*, and in two other presumptive human/hamster hybrid tumours established *in vivo*¹⁰, raises the question of whether this might represent a marker chromosome for such tumours in the hamster, and not necessarily be of human derivation. We are cognisant that rearrangements of hamster chromosomes could have resulted in banding patterns similar to those of the chromosomes identified as human by this morphological method.

The frequency of spontaneous cell fusion *in vitro* has been reported to be very low for most cell types combined, and has been estimated to occur at 1×10^{-6} to 5×10^{-6} (ref. 14). The increased frequency of somatic cell fusion induced with the aid of myxoviruses^{15,16} raises the possibility that the *in vivo* hybridisation suggested in this human tumour transplantation and in previous studies²⁻⁷ may have been influenced by tumour- or host-borne fusing agents.

It is usual for many somatic cell hybrids to lose selectively the chromosomes of one of the parents while the chromosomes from the other remain dominant in number. In the case of human/rodent cell combinations, it is the human chromosomes which are preferentially lost¹⁷⁻¹⁹. Although this is the only reported case of a putative *in vivo* heterospecific hybridisation involving karyological analysis of the hybrid progeny, it seems that a number of relationships found in cell fusion *in vitro* are true for the *in vivo* situation. Our human/hamster cell fusion results indicate that pronounced chromosomal segregation occurs following hybrid formation, where the human chromosomes are preferentially lost. Further, malignancy, when associated with the human parental cells which experienced chromosomal loss, can be expressed in the hybrid progeny, even after a relatively long sojourn in the animal host.

The animal host would be expected to eliminate any human chromosomes in the hybrid tumour cells, particularly if these could restrict the hybrid tumour's viability. Yet, human chromosomes seem to have been retained in the hybrid tumour for at least up to 15 serial transplant passages, or a minimum of 18 weeks. Whether or not the portion of the human genome controlling malignancy required the retention of any particular human chromosomes or chromosomal fragments for the expression of malignancy in the hybrid, or the loss of some inhibitory genes, cannot be concluded on the basis of these data. It is nevertheless intriguing to follow the chromosomal constitution of a human/hamster hybrid tumour during extensive propagation in hamsters, and to explore to what extent the human genome is functional and being recognised by the hamster host.

As *in vivo* hybridisation seems to have permitted a human tumour to become adapted and unusually lethal in the hamster, we suggest that similar mechanisms may be implicated in human cancer, particularly when such fusing agents as parainfluenza viruses are prevalent in man. In the autochthonous tumour-

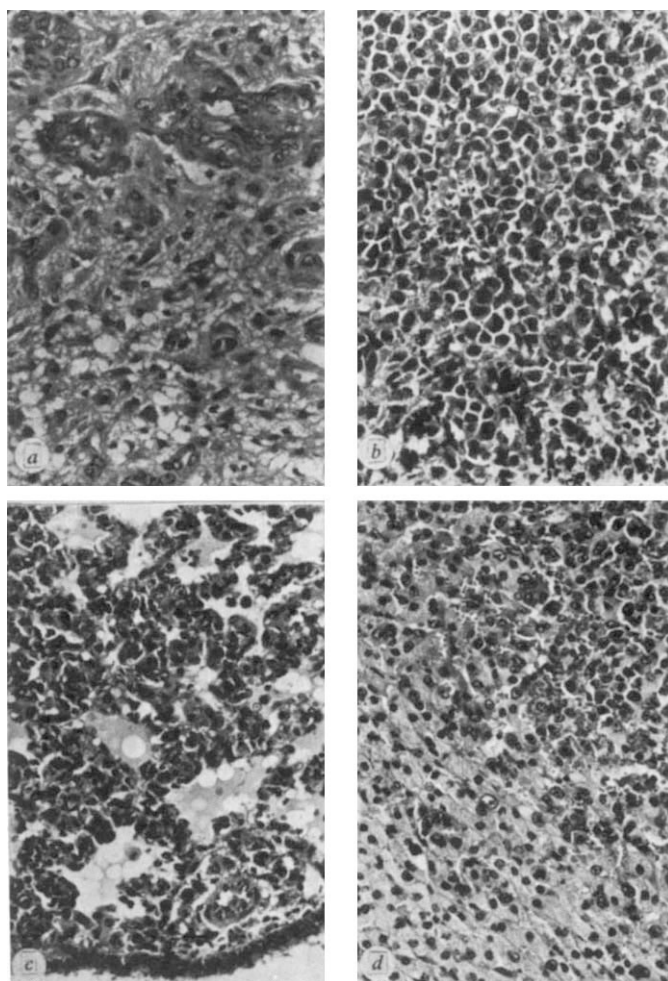


Fig. 1 Microscopic morphology of original human cerebral tumour before heterotransplantation and of the human/hamster hybrid tumour, GB-749 (hematoxylin and eosin, $\times 208$). *a*, Patient's poorly differentiated astrocytic glioma showing pleomorphic tumour cells and a proliferation of the vascular endothelium; *b*, GB-749 tumour cells growing in the hamster cheek pouch; *c*, GB-749 metastasis from hamster cheek pouch to lungs; *d*, subcapsular focus of GB-749 tumour cells in adrenal 4 weeks after transplantation to the cheek pouch.

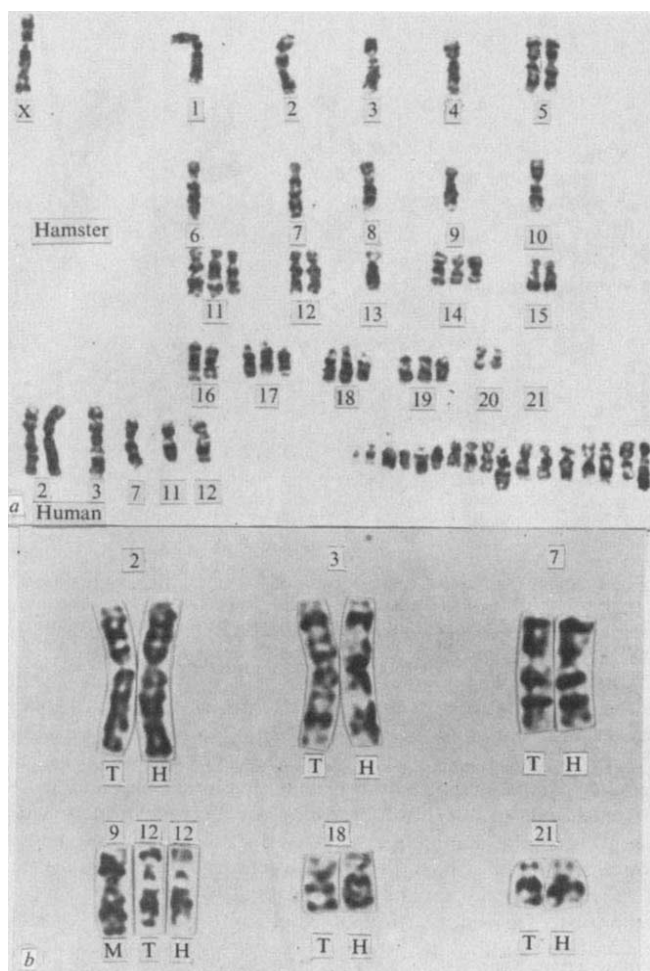


Fig. 2 a, Karyotype of gicmsa-banded chromosomes of a GB-749 tumour cell showing a simultaneous presence of hamster-like, human-like, and a series of unidentifiable chromosomes (lower right); b, Comparison of banded chromosomes selected from several GB-749 tumour cells (T) and the cancer patient's peripheral lymphocytes (H). Human chromosome No. 12 in GB-749 (T) and in the patient's blood (H) are also compared with hamster chromosome No. 9 (M).

bearing host, fusions of neoplastic with normal cells might provide the tumour with a means for reducing any tumour-specific antigenicity and thereby allow it to escape immunological surveillance mechanisms. In these terms, tumour progression by fusion would permit the neoplasm to circumvent the host's immunological controls. Conversely, fusing the tumour with other cells *in vitro* may provide a more suitable immunogen for the evocation of an immunological reaction against a tumour of advanced malignancy. Although still speculative, such considerations may offer other avenues for the immunological control of cancer.

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Evolution of X-chromosome inactivation in mammals

It is now well established that in the somatic cells of mammals only a single X chromosome is active in coding for proteins, no matter how many are present^{1,2}, but the mechanism and evolutionary origin of this phenomenon still remain unsolved problems. Cooper³ suggested that the random inactivation of X chromosomes derived from either parent in eutherian mammals had evolved from a more primitive inactivation of the paternally derived X chromosome as seen in marsupials, and a possible mechanism for this evolution has been put forward⁴. Lifschytz and Lindsley⁵ further suggested that the inactivation seen in somatic cells had evolved from inactivation of both sex chromosomes in male gametogenesis, a phenomenon that is general in gametes of the heterogametic sex of many species.

All of these proposals have obvious merit, but in detail criticisms can be levelled at all. It may therefore help to consider the possible evolution of X chromosome inactivation more fully. It is reasonable to suppose that mono-allelic activity of the X chromosome is connected in some way with its function as a sex chromosome. In germ cells of normal females both X chromosomes are clearly active⁶⁻⁸. Moreover, they are required to be so for normal ovarian function in both human and mouse^{9,10}. In males, if more than one X chromosome is present, as in XX and XXY males of various species, the germ cells die at the spermatogonial stage^{2,11}. The explanation that this is due to activity of all X chromosomes in male germ cells as well as female has recently been confirmed by the finding that loss of an X chromosome enables germ cells to survive in these animals¹². But, as previously mentioned, later in male gametogenesis the X seems to become inactive. Thus, the cycle of X chromosome activity is: somatic cells, single; female germ cells, double; late male germ cell, none.

Thus, the X chromosome clearly has a role in the germ cells. By contrast, sex determination in mammals is effected through the Y chromosome. In the presence of the Y chromosome the embryonic gonad is induced to become a testis¹³.

The testis then secretes testosterone which, in combination with the product of a certain gene, activates all genes required for male differentiation, so that the animal develops as a normal male¹⁴. In the absence of the Y the gonad becomes an ovary, testosterone is not secreted, and differentiation follows the female pattern. Thus, there is a simple switch mechanism controlling sex determination and

differentiation¹⁴. Mutation of the gene involved in response to testosterone leads to the syndrome of testicular feminisation, in which a genetic male has testes but no other signs of maleness, and fails to respond to testosterone. In the mouse the locus of this gene, called the *Tfm* gene, is on the X chromosome¹⁵. Hence, by Ohno's law of the conservation of the mammalian X chromosome¹⁶ it is probably X-linked in all mammals, and thus gives another function of the X chromosome in sex differentiation.

The sex chromosomes of present day mammals are typically clearly distinguishable, with a small Y and fairly large X, the variably active part of the X constituting roughly 5% of the genome¹⁶, although some species have other material (constitutive heterochromatin or autosomal material) attached to the X. By contrast, the sex chromosomes of lower vertebrates are commonly not distinguishable¹⁶. Thus, in evolution the mammalian Y must have lost material, and the X has perhaps gained some.

Concurrently, sex determining processes have evolved. Lower vertebrates respond to hormones in a way comparable to the mammalian response to testosterone, but in contrast to mammals sex determination is more labile, and sex reversal can be achieved by hormone treatment. In view of the response to testosterone one may suppose that a gene comparable to the *Tfm* gene was present early in evolution, before the sex chromosomes became morphologically distinct. The next stage was the formation of a small differential segment, containing in the Y the genes involved in testis formation. At this stage, the locus of the *Tfm* gene will have been present on both sex chromosomes, and genes concerned in ovarian function will have been distributed over both X and Y, interspersed with many genes not directly involved in sexual development (homologues of present X-linked genes). After this, the Y chromosome lost much of its material, including the homologues of the X-linked genes. There was then a dosage difference between males and females, which was corrected by X chromosome inactivation in somatic cells.

It is however difficult to envisage exactly how this inactivation first arose. As seen at present it is an all-or-none phenomenon. The inactivated chromosome (except for a possible small region escaping inactivation) is totally and irreversibly unresponsive to stimuli evoking transcription, and it seems unlikely that it could have evolved to this state by a gradual process. There seems no intermediate stage. But total inactivation of one X chromosome when previously two had been active would in effect result in functional monosomy for X-linked genes and monosomy in mammals is usually lethal¹⁷. Thus, the evolution of X inactivation would be easier to envisage if some duplication of genes had occurred first. This could have been achieved

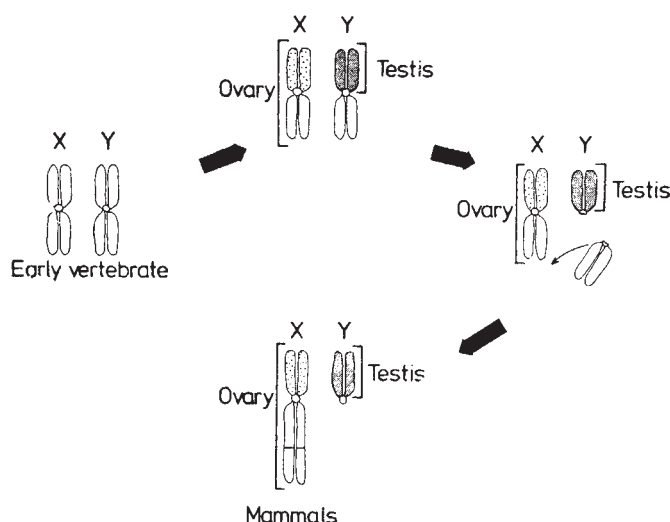


Fig. 1 Suggested evolutionary origin of mammalian sex chromosomes. The position of the centromere, and the relative lengths of the dotted, shaded and white regions are purely diagrammatic and not to scale. The white regions are those postulated to be subject to inactivation in present mammals.

if the material lost from the Y chromosome had been transferred to the X, giving in effect a tandem duplication of the major part of the original X chromosome (Fig. 1). Female offspring which received such a duplicated X chromosome, either homozygously, or heterozygously with the original, would have excess X-linked genes, and hence would probably not be viable, unless the excess material were inactivated. Thus, one must also postulate that a mechanism for inactivation of X chromosomes had already evolved before the proposed Y-X transfer occurred. This, one must suppose, was the form of inactivation seen in late male germ cells, with the mechanism now modified so that it operated in somatic cells, but only on excess material. Such a system could have been selectively advantageous if the additional X chromosomal material was active and beneficial in female germ cells.

From this hypothesis of the evolutionary origin of X-chromosome inactivation at least two predictions can be made. One is that there should be a small region of the present mammalian X chromosome which is not subject to inactivation. This would be the original differential segment of the X, which by definition could have had no homologue on the Y. Therefore, it could not have been transferred from the Y to the X, and hence was never present in excess.

This does indeed seem to be the case in the human X chromosome. The Xg blood group locus is apparently not subject to inactivation in a normal X chromosome¹⁸. Moreover human XO individuals have a range of malformations, which seem to be due to deficiency of gene(s) located in the short arm of the X¹⁹ and in individuals with supernumerary X chromosomes the finger-ridge count, the stature and mental ability seem to vary additively with number of X chromosomes present^{9,20}. All these facts could be explained if a part of the short arm of the human X chromosome was not subject to inactivation, because it represented the original differential segment. It is not clear, however, whether such a region exists in other species. Chromosomally XXY males are known in a number of species and no clear pattern of abnormalities, except sterility, emerges². Females with the XO constitution are known in the pig²¹, the rhesus monkey²², and the cat²³ and in each there was at least one abnormality also seen in human XOs. But in XO animals of several rodent species (mouse, black rat *Rattus rattus*²⁴, *Akodon*²⁵ and *Microtus oregoni*²⁶) no somatic abnormalities were noted, and some were fertile. Possible explanations for an apparent lack of

Table 1 Human X-linked diseases existing in more than one form

Probably at least two gene loci		
Colour blindness	30380 Deutan	30390 Protan
Haemophilia	30670 Haemophilia A	30690 Christmas disease
Muscular dystrophy	31010 Becker	31020 Duchenne
Retinal degeneration	31270 Retinoschisis	31060 Norrie's disease
Either alleles or multiple loci		
Adrenal cortical defect	30010	30020
Agammaglobulinaemia	30030	30040
Amelogenesis imperfecta	30110	30120
Cerebral sclerosis	30270	31160
Charcot-Marie-Tooth disease	30280	30290
Congenital cataract	30220	30230
Deafness	30440	30450, 30460, 30470
Diabetes insipidus	30480	30490
Ocular albinism	30050	30060
Retinitis pigmentosa	30310	30320, 30330, 31260
Thrombocytopenia	30100	31390, 31400

The numbers are the catalogue numbers given by McKusick²⁹.

the original differential segment in rodents are that in the course of evolution it has been transferred to an autosome, or has been duplicated and then become subject to inactivation.

A second and important prediction is that there should be some evidence of duplication of gene loci on present mammalian X chromosomes. It is to be expected that during evolution the originally duplicate genes will have mutated and diverged, but some evidence of similarity should still remain. Only the human and the mouse X chromosomes are genetically at all well known, the human having far more known X-linked genes than the mouse. McKusick²⁷ pointed out that several X-linked diseases exist in more than one form. The various forms could either be due to different mutant alleles at the same gene locus, or to the involvement of more than one locus for each disease. This point can only be tested conclusively by chromosome mapping. From the latest evidence²⁸ it is highly probable that there are at least two gene loci involved in each of four X-linked diseases: colour blindness (protan and deutan), haemophilia (A and B), muscular dystrophy (Duchenne and Becker), and retinal degeneration (retinoschisis and Norrie's disease) (Table 1). In addition, there are at least eleven other diseases listed by McKusick²⁹, which exist in more than one form, and hence are possibly caused by duplicate loci. McKusick lists 150 X-linked conditions, 86 of which he considers well established. This must be only a small fraction of the probably several hundred genes on the human X chromosome. Hence it is hardly to be expected that both members of every pair of duplicate genes would have been discovered, and the present evidence must be considered quite good. The weakest point is that the similarity between pairs of diseases has been judged at the clinical level, and molecular evidence for the similarity or otherwise of the proteins involved is lacking. By contrast, in the mouse only about 28 X-linked genes are known, thought to be at about 21 loci³⁰, and there is as yet no evidence of duplication.

Thus, the supporting evidence from man is sufficient to make the hypothesis of evolution of X chromosome inactivation through Y-X transfer well worthy of further consideration. To reiterate, the hypothetical sequence of events was: (1) formation of a small differential segment, carrying testis-forming genes in the Y, from previously undifferentiated sex chromosomes; (2) inactivation of the sex chromosomes during male gametogenesis, but full activity of both homologues in female germ cells; (3) transfer of a large part of the homologous region of the Y to the X, and concomitant alteration of the inactivation process, so as to inactivate all excess X chromosomal material in somatic cells, while retaining full activity in female and early male germ cells, and complete inactivity in late male germ cells; (4) slow divergence of the two halves of the duplicated X by mutation and selection.

Further evidence could be obtained from studies of X chromosome anomalies in a range of mammalian species. Human X chromosome linkage data could be re-examined with the possibility of duplicate loci in mind, and at the molecular level proteins coded by X-linked genes could be studied for evidence of duplications.

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A possible role for prolactin in control of steroid secretion by the human Graafian follicle

PROLACTIN constitutes part of the luteotrophic complex necessary for the maintenance and secretory activity of the corpus luteum in the rat^{1,2}, mouse³, rabbit⁴, hamster⁵, ferret⁶, pig⁷ and sheep⁸. Recent evidence suggests, however, that prolactin may have little to do with luteal function in women⁹⁻¹⁵. Nevertheless, it is still possible that prolactin could play a 'permissive' role, as in other species, where alterations in peripheral blood levels, within limits, have little effect on luteal activity. In an attempt to gain more insight into the possible role of prolactin in controlling ovarian activity in women, we have studied the production of steroids by human granulosa cells growing in tissue culture, and the effects of the addition or neutralisation of human prolactin in the culture media. We report that the production of progesterone by human granulosa cells *in vitro* requires low physiological concentrations of prolactin whereas high concentrations are inhibitory.

Ovaries, endometrial biopsies and peripheral blood samples were obtained from patients aged 21-48 yr who were undergoing surgery for gynaecological disorders. The stage of the menstrual cycle was assessed from the date of the last menstrual period, the concentrations in plasma of luteinising hormone (LH), follicle stimulating hormone (FSH) and progesterone, endometrial histology, and the presence or absence of a corpus luteum. Only follicles from morphologically normal ovaries were used as the source of granulosa cells in this study.

Antral follicles were dissected out of the ovary within 2 h of ovariectomy and the fluid aspirated by syringe. The follicular fluid and peripheral plasma sample were stored at -20°C until assayed for prolactin and progesterone by

specific radioimmunoassays (RIA). Prolactin concentration is expressed as ng ml^{-1} Friesen prolactin of which $1 \text{ ng} \equiv 20 \mu\text{U}$ MRC Standard 71/222. The Friesen prolactin preparation was 90% pure and the human pituitary hormone contaminants were found by RIA to be negligible: growth hormone (HGH), HLH, HFSH, all $<0.4\%$ and the concentration of human placental lactogen (HPL) undetectable. The collapsed follicle was slit open and the granulosa cells scraped into culture medium. An aliquot of the cell suspension was counted and the viability determined¹⁶. The follicle wall was placed in fixative for subsequent histological examination to ensure that the basement membrane was still intact. The cells were layered on to 18 mm^2 coverslips and cultured for 10–14 d as previously described¹⁷. The culture medium was replaced every 24 h and stored at -20°C until assayed for progesterone. The variation ($\pm \text{s.e.m.}$) in the production of progesterone between replicate cultures for all treatments was found to be 6.77 ± 1.45 .

Neutralisation of prolactin in the culture medium by the addition of rabbit anti-human prolactin serum, at a dilution which did not cross react with HPL, HGH, HLH or HFSH, caused a significant decrease in progesterone production as compared to the cultured control (Fig. 1). To

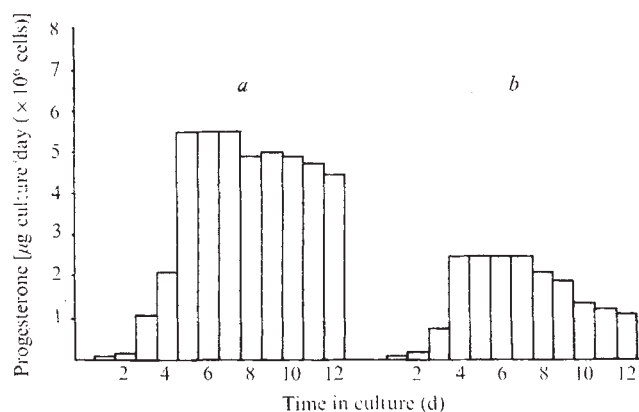


Fig. 1 Progesterone production by luteinised granulosa cells in a culture medium where the endogenous prolactin was neutralised with anti-human prolactin serum. The cells were collected from a single late follicular phase preovulatory follicle. The concentration of endogenous prolactin in the culture medium before the addition of rabbit anti-human prolactin serum was 5.1 ng ml^{-1} . After precipitation of the prolactin-anti-prolactin complex the concentration was $<0.2 \text{ ng ml}^{-1}$. The concentrations of progesterone in the control were determined with either normal rabbit serum, or rabbit anti-bovine serum albumin added to the culture medium at the same dilution (1/500) as the antiserum used in the anti-rabbit culture treatment. There were no significant differences in the production of progesterone between the control culture which were found to be within the precision achieved in replicate cultures. *a*, Control; *b*, anti-prolactin, $P < 0.01$.

confirm that free prolactin in the culture medium had been completely neutralised by the antiserum, the anti-prolactin-prolactin complex was precipitated out using a solid phase second antibody; the concentrations of LH and FSH in the medium were unaltered whereas prolactin was undetectable.

Addition of human prolactin to the culture medium had no effect on progesterone production if the final prolactin concentration did not rise above 20 ng ml^{-1} ; the mean concentration of prolactin in the peripheral blood of women during the cycle is 15 ± 1 ($\pm \text{s.e.m.}$)¹⁰. When the concentrations in the culture medium were increased from 25 to 100 ng ml^{-1} , there was a progressive decrease in the daily progesterone production (Fig. 2). This inhibitory effect persisted, even when the LH and/or FSH concentrations

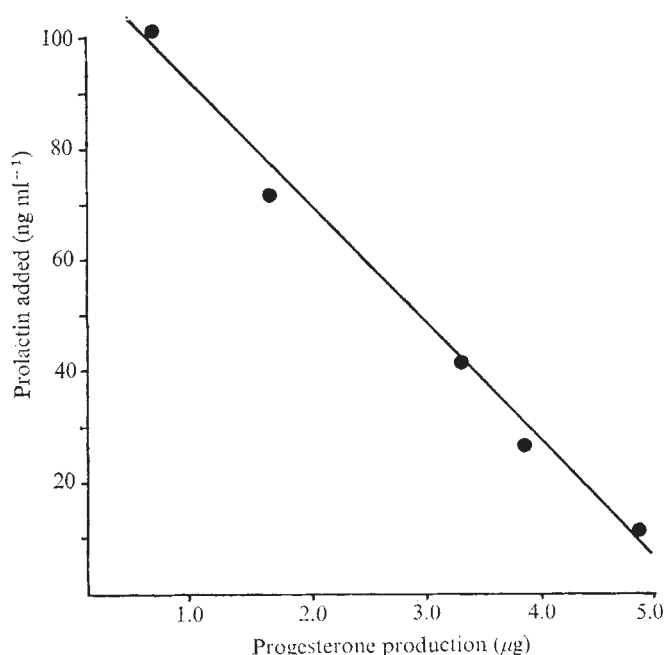


Fig. 2 Relationship between the concentration of prolactin and the total progesterone production by human granulosa cells *in vitro*. Granulosa cells were collected from one late follicular phase preovulatory follicle. Each point is the mean result of duplicate cultures. Total progesterone production is that achieved by 10^6 granulosa cells in 11 consecutive daily changes of culture medium.

were increased 50-fold, and it could be obtained with granulosa cells collected from follicles at any stage of the menstrual cycle. In contrast the soluble non-protein fraction of the added prolactin solution did not inhibit the production of progesterone by human granulosa cells *in vitro* as compared with the controls.

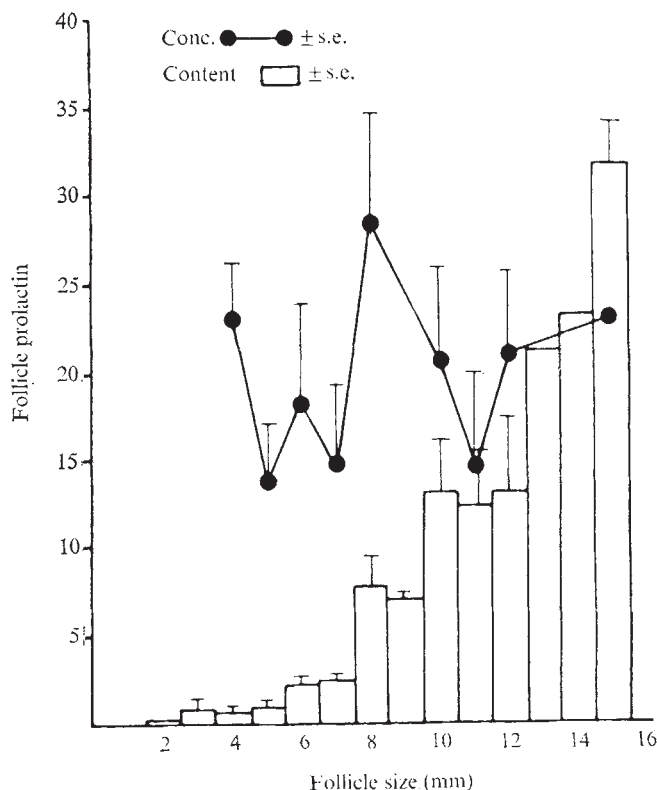


Fig. 3 Changes in concentration and content of prolactin in follicular fluid with respect to follicle size. Vertical bars represent 1 s.e.m. Circles show prolactin concentration (ng ml^{-1}); bars show prolactin content (ng).

Prolactin was assayed in 110 samples of follicular fluid collected at various stages of the cycle. The mean concentration ($\pm$ s.e.m.) was 20 ± 5 ng ml⁻¹ for all sizes of follicle. The increase in the prolactin content of the follicle was correlated with the volume of follicular fluid (Fig. 3). The prolactin concentrations during the late follicular phase of the cycle were, however, significantly lower than at any other time of the cycle with the exception of the early luteal phase (Fig. 4).

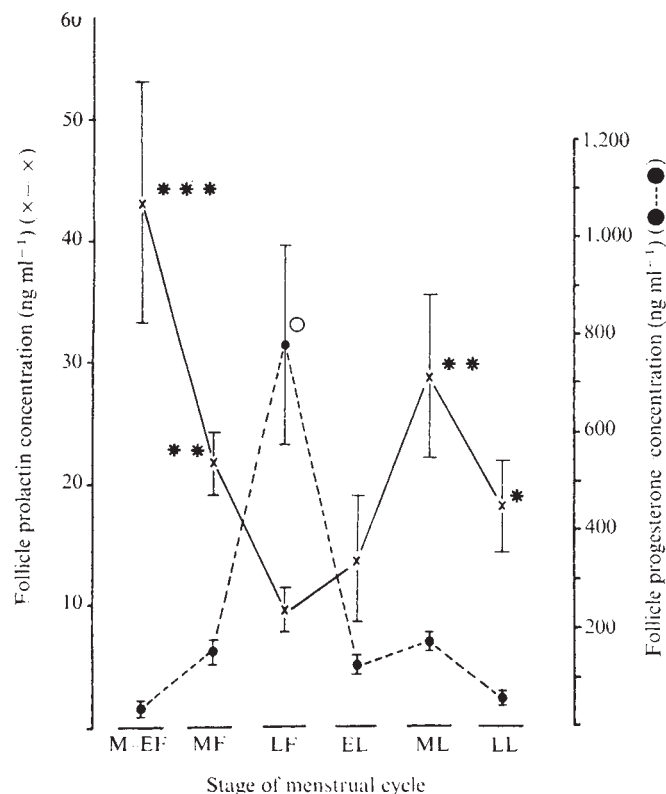


Fig. 4 Changes in the follicular fluid concentrations of prolactin and progesterone related to the stage of the menstrual cycle. The vertical bars represent 1 s.e.m. (M-EF; MF; LL; represent menstruation-early follicular, mid follicular and late follicular phases, and EL, ML and LL represent early, mid and late luteal phases.) *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; ° $P < 0.001$.

These *in vitro* experiments suggest that high concentrations of prolactin within the follicular fluid may actually depress progesterone secretion by the granulosa cells. This might provide an explanation for the fact that galactorrhoea with high prolactin levels is commonly associated with amenorrhoea¹⁸, and that if the prolactin levels are lowered by treatment with CB 154, ovulation and menstruation recur^{19,20}. It is well recognised that post-partum lactation can inhibit ovulation in women and other animals^{18,21}, although the mechanism has never been explained.

Although there are now numerous descriptive accounts of the changing hormone levels in blood and urine throughout the menstrual cycle, we do not really understand what factors are responsible for the formation, maintenance and regression of the human corpus luteum. This is an area of great potential interest for the development of new forms of contraception. The *in vitro* results presented here cast doubt on the simplistic view that LH is the only gonadotrophin necessary for luteal maintenance¹⁵, and suggest that prolactin is also involved. Furthermore, it seems probable that the gonadotrophic content of the follicular fluid may have important consequences for the secretory activity of the granulosa cells both before and after ovulation.

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Anatomy of an identified serotonin neurone studied by means of injection of tritiated 'transmitter'

THERE is an identifiable serotonin-containing neurone in each cerebral ganglion of the snail *Helix pomatia*¹. Electrophysiological evidence suggests that each neurone sends a process into each cerebro-buccal connective^{2,3}, and that monosynaptic connections are made in each buccal ganglion with other large identifiable neurones³. Because this neuronal system seems to offer a unique opportunity to study the proposed transmitter role of serotonin^{3,4}, it is important to obtain direct information on the localisation and morphology of the presynaptic endings of the serotonin cell. This is not possible with dye⁵ or metal ion⁶ injection techniques, which do not seem to mark processes of the

serotonin neurone at distances of more than a few millimetres from the neurone perikaryon.

In this study we injected tritiated serotonin, or its precursor 5-hydroxytryptophan, into the perikaryon of one of the identified symmetrically placed, serotonin neurones and, after allowing time for the radioisotope to pass along the processes of the neurone, the tissue was prepared for light and electron microscope autoradiography.

The tips (diameter 1 to 3 μm) of glass microelectrodes were filled with a solution of uniformly tritiated serotonin (5-hydroxytryptamine creatinine sulphate) or 5-HTP (DL-5-hydroxytryptophan) (Radiochemical Centre, Amersham). Solutions were prepared by evaporating to dryness 1 mCi of radioactive solution and redissolving in a small volume (0.01 ml) of distilled water. (The isotope specific activities were: serotonin 11 Ci mmol^{-1} and 5-HTP 7 Ci mmol^{-1} ; concentrations of the injection solutions were approximately 0.9 mM and 1.4 mM, respectively). Electrodes were selected by measuring isotope release from the tip into 0.2 ml of snail physiological solution⁷ after passing 10 nA positive square wave pulses of 100 ms duration at 5 Hz for 10 min. Electrodes releasing sufficient isotope to yield 5,000 to 20,000 c.p.m. (measured in a total volume of 10 ml including 9.8 ml of scintillation fluid, counted on a Packard Liquid Scintillation Spectrometer) were found to be suitable for intracellular iontophoresis.

Either the left or right giant serotonin neurone was impaled and positive pulses applied to the electrode for 1 to 2 h; generally 10 to 20 nA pulses of 100 ms duration at 5 Hz were used. The preparation was continuously washed with physiological solution. The direction of flow was from the buccal ganglia and lip nerves towards the cerebral ganglia, thus avoiding contamination of the areas suspected of containing processes and endings of the neurone. During the period of injection, the resting potential of the neurone decreased to between 0 and 10 mV, but

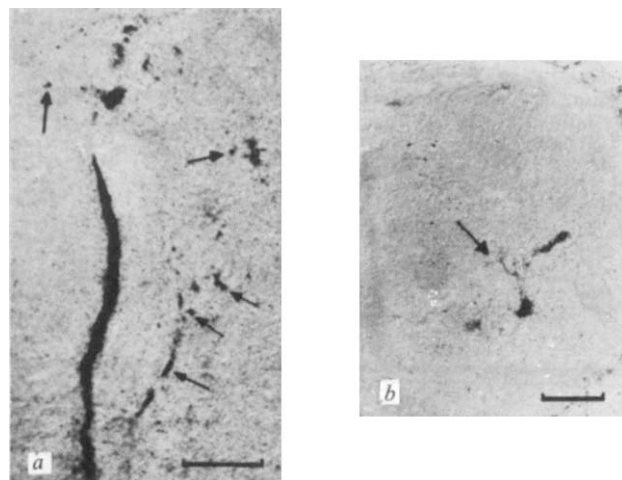


Fig. 1 Micrographs of autoradiograms of a right giant serotonin neurone injected with tritiated serotonin. The areas shown in *a* and *b* are indicated in Fig. 2. *a*, Axon of the serotonin neurone which runs in the contralateral cerebral ganglion. Approximately 25 small branches of this axon (some arrowed), which are shown in different degrees of oblique and transverse section, can be recognised as dark spots. The two larger dark spots at top and right are cross sections of the main axons which run into the lip nerves. In *b*, the largest dark spot is the main axon of the serotonin cell in the ipsilateral buccal ganglion cut in transverse section. Branches of this axon (one fine branch arrowed) terminate within the buccal ganglion neuropile, which is in the middle of the photograph. Scale bars represent 25 μm .

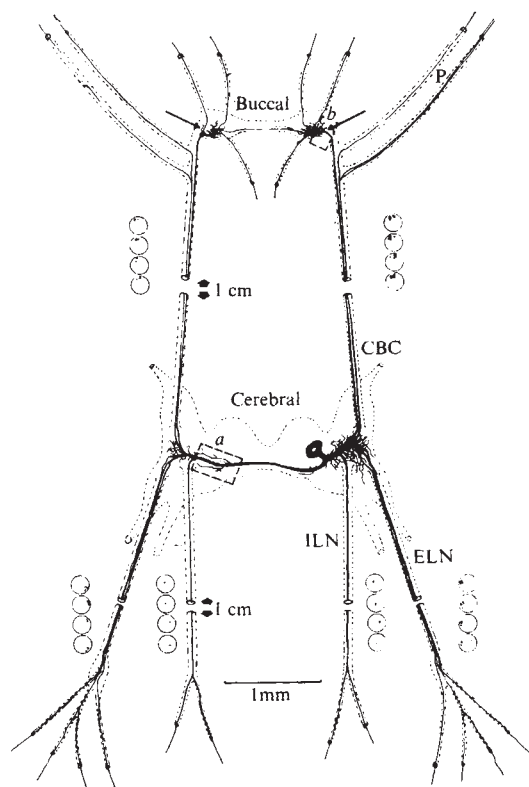


Fig. 2 Diagram of the processes of a right giant serotonin neurone injected with tritium-labelled serotonin. The cerebral and buccal ganglia are joined by connective nerve trunks (CBC). The position of identified neurones in each buccal ganglion which receive synaptic input from the serotonin neurone is indicated with an arrow. Axon branches pass close to, and there are numerous small terminal processes in the immediate vicinity of, these follower neurones. Each cerebro-buccal connective contains two axon processes of the serotonin cell which run close together within the connective. Axon processes pass into the nerves supplying the buccal mass and into the pharyngeal nerves (P), which supply the muscles which control feeding. Two or three separate axon processes of the serotonin neurone pass into each external lip nerve (ELN). The axon processes within these nerves run to muscles in the mouth of the animal. It thus seems that all the peripheral axon branches of the serotonin neurone are somehow associated with feeding. The size and depth of the axon processes observed in transverse sections of each nerve are relatively constant. This is indicated by the diagrams of the cross sections of the nerve trunks, where each circle beside a particular trunk illustrates the situation in a different ^3H -serotonin injected preparation. The processes are located close to the periphery of the CBC and ELN but centrally within the internal lip nerve (ILN). The axon processes of the serotonin neurone are not round in cross section, but have infoldings which vary in shape from section to section. The many small branches within the right and also possibly the left, cerebral ganglia, are thought to be dendrites receiving synaptic input. The areas indicated by *a* and *b* are illustrated in Fig. 1.

cells partly recovered after injection (the resting potential returned to about -20 to -40 mV). The preparation was left for 10–15 h at 4°C before processing for autoradiography.

For light microscope autoradiography, the tissue was fixed for 2 h in 2.5% glutaraldehyde solution, dehydrated in a series of ethanol solutions and embedded in wax. Radioactivity was monitored in the histological solutions. Little if any wash out of the isotope could be detected, that is, less than 100 c.p.m. ml^{-1} of any histological solution. Serial sections (8 μm) were prepared and mounted on glass slides. The sections were covered with Kodak AR 10 stripping film and developed in Kodak D 19 after storage in the dark for

two weeks. For electron microscope autoradiography, tissue was fixed in glutaraldehyde and osmium solutions and embedded in Epon. Thin sections were covered with a monolayer of Ilford L4 emulsion by a loop technique⁸, exposed for three weeks, and subsequently developed in Kodak Mikrodol-X.

Similar results were obtained in all of the experiments. Five light microscope experiments (three left and one right serotonin neurones were injected with ³H-serotonin and one right neurone was injected with ³H-5-hydroxytryptophan) and two electron microscope experiments (two left serotonin neurones injected with ³H-serotonin) were made. The radioactive material was sufficiently well bound to withstand the histological treatment and it was readily detected in the autoradiograms (Figs 1 and 3). Apart from the region in the immediate vicinity of the perikaryon of the serotonin neurone, where there was some spillage and binding of the isotope to connective tissue, it appeared that all the radioactivity detected in the autoradiograms was strictly located within the giant serotonin neurone. The main axons could be easily followed in serial sections with the light microscope and fine terminal branches could be traced at a distance of 1 cm or more from the perikaryon, for example in the buccal ganglia (Fig. 1b), where there was a complete lack of silver grains over the outer connective tissue capsule. The processes of the right serotonin neurone traced by this method are shown diagrammatically in Fig. 2. The processes of the left serotonin neurone form a pattern which is the mirror image of the right neurone.

The results of the light microscope autoradiography showed that axon processes of each giant serotonin neurone pass to the region of the buccal ganglia where the identified follower neurones are located. In these regions many fine terminal branches were seen. In some sections, silver grains were seen directly adjacent to the axon hillocks of the identified follower neurones. This morphological evidence therefore agrees with the electrophysiological results suggesting the presence of monosynaptic connections of the serotonin neurones and these follower neurones. The distribution of fine branches in the neuropile of each buccal ganglion was very similar to that observed after uptake of exogenous ³H-serotonin by the isolated buccal ganglia⁹.

The pattern of processes was remarkably similar in each preparation. The presence of two or more axon processes running parallel for considerable distances along some nerve trunks is an interesting, but so far unexplained phenomenon, also noted by other workers¹⁰. There was a constancy in the relative sizes of the different processes within a particular nerve trunk and their positioning with respect to the centre or periphery of the particular nerve trunk (see Fig. 2). It is apparent that there is an extensive overlapping of the fields of innervation of each giant serotonin cell, as was suggested by electrophysiological experiments^{3,4}.

When a cerebro-buccal connective or external lip nerve was ligatured, there was a build up of radioactivity on the side of the constriction nearer the perikaryon. This was shown by an increase in density of silver grains within the axon of the serotonin cell against the ligature. This experiment suggested that the labelled serotonin was actively transported along the axons. The minimum rate of movement of the isotopically labelled material within the serotonin cell axon was 1.0 mm h⁻¹.

By electron microscope autoradiography it was possible to study the axons of the serotonin neurone within the cerebro-buccal connectives and some of its terminal processes within the neuropile of the buccal ganglia (Fig. 3). The identification of groups of silver grains over processes of the serotonin neurone was comparatively easy, because after the relatively short time periods of radiographic exposure employed, background silver grains were almost completely absent. Identification of processes from the serotonin neurone with the electron microscope was also

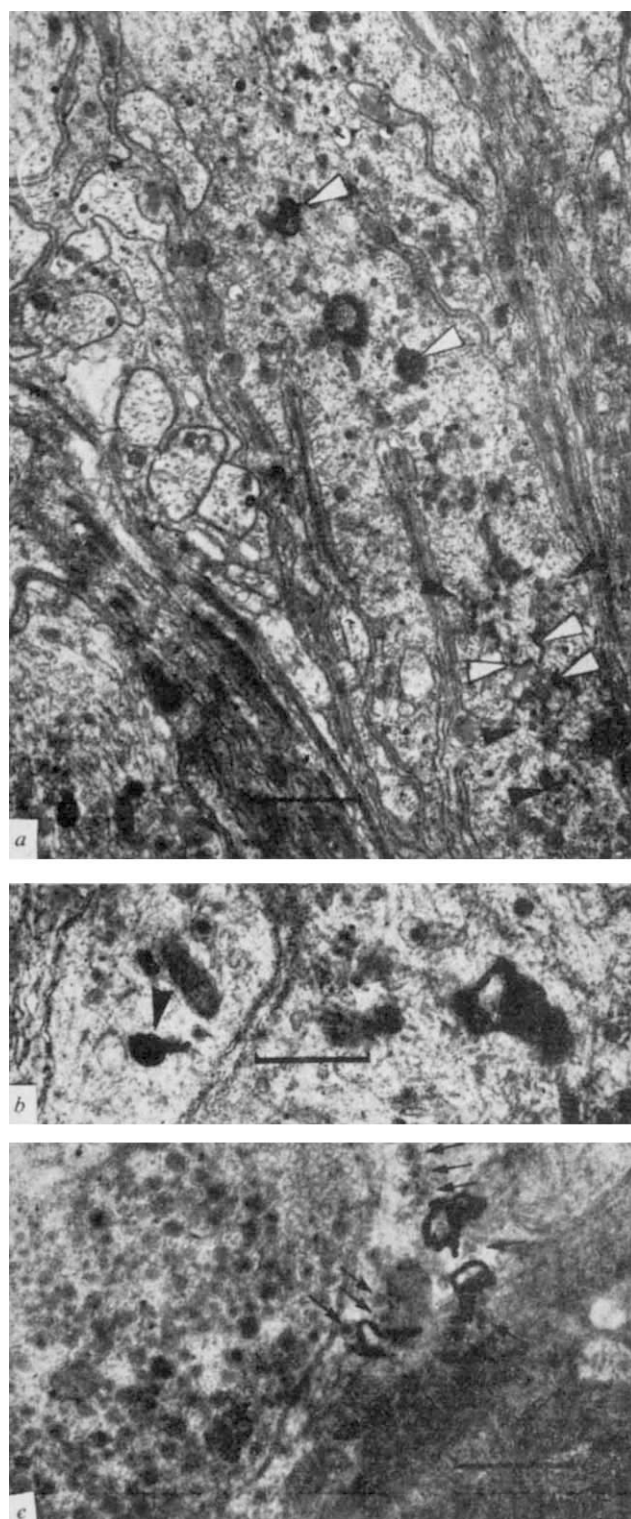


Fig. 3 Electron microscope autoradiograms of cross sections of the cerebro-buccal connective and buccal ganglia showing axons of a serotonin neurone injected with tritium-labelled serotonin. In *a* the silver grains are marked with white arrows for clarity; the black arrows point to groups of dense-cored vesicles. In *b* the black arrow points to a silver grain which overlies a dense-cored vesicle. The silver grain to the right in *b*, and also the silver grain second from top in *a*, seem to be associated with larger electron-dense structures. *c*, Presumed terminal process or an injected serotonin neurone within the neuropile of a buccal ganglion. The arrows point to dense-cored vesicles. The star marks a silver grain which does not obviously overlie the same structure as the other silver grains, although it is possible that this is the case, and that the labelled ending is shown at two levels within the same section. Scale bars represent *a*, μm ; *b*, $0.5 \mu\text{m}$; *c*, $0.5 \mu\text{m}$.

aided by comparison with adjacent thicker tissue sections processed for light microscope autoradiography.

All the labelled axon processes contained vesicles with electron-dense cores (Fig. 3). These vesicles had an average diameter of 100 nm, and were morphologically identical to those which are thought to sequester serotonin in the perikaryon of the serotonin neurone¹¹ and those seen in a small proportion of nerve endings in the buccal ganglia which selectively accumulate exogenous tritium-labelled serotonin⁸. Some silver grains overlay such vesicles (Figs 3b and c).

None of the terminal processes observed resembled classical synapses in containing membrane thickenings, however 'typical' synapses are only rarely seen in the central nervous system of this animal¹¹. Because the fine terminal processes of the serotonin neurone in the neuropile regions contain many vesicles (Fig. 3c), they probably represent, or are close to, the release sites of the transmitter.

The results suggest that the technique of injecting tritiated 'transmitter', or a readily metabolised precursor, may be of general value in mapping the axons, and locating and studying the structure of the presynaptic terminals of individual neurones of known transmitter type. The technique is similar to that of injecting radioactive amino acids before autoradiography, which has been shown to be valuable in studying the anatomy of, and axonal transport within, the dendrites of single mammalian spinal motor neurones^{12,13}. In the latter situation, however, there was evidence for a transfer of radioactivity between glia and other neurones, and it was not established that any of the proteins presumed to be labelled after incorporation of the radioactive amino acids might have been destined for the presynaptic terminals^{12,13}. On the other hand, it might be expected that injected transmitter will be treated as natural transmitter and transported to and concentrated within presynaptic terminals. (Some experimental results, not described here, obtained after the injection of leucine into identified snail neurones support this view). Further, the results show that no noticeable damage is caused by the iontophoresis of tritium labelled serotonin to the fine structure of the neurone. This is not the case with other intraneuronal staining techniques^{3,6,14,15}. Finally, the results show for the first time that the presynaptic endings of a specified neurone of known transmitter type can be studied in direct relation to its perikaryon.

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Calcium ionophore X-537A increases spontaneous and phasic quantal release of acetylcholine at frog neuromuscular junction

TRANSMITTER release from nerve endings is believed to be triggered by the influx of Ca^{2+} , which is thought to enter the terminal as a result of an increase in conductance produced by the action potential¹. At the neuromuscular junction the evidence for the role of Ca^{2+} is largely indirect, based on changes in endplate potential (e.p.p.) amplitude following variations in $[\text{Ca}^{2+}]_0$. Miledi² showed that microinjection of Ca^{2+} into the presynaptic terminal at the synapse of the squid giant axon causes a brief period of enhanced quantal release. Ca^{2+} also affects the rate of spontaneous quantal release at the neuromuscular junction; miniature end-plate potential (m.e.p.p.) frequencies are very low in solutions with reduced Ca^{2+} (ref. 3-5). The mode of action of Ca^{2+} within the terminal remains largely a matter of speculation. Now a new tool has become available: an ionophore, X-537A, that transfers both univalent and divalent cations across lipid bilayers and cell membranes⁶⁻¹⁰. Using X-537A it may be possible to change intracellular Ca^{2+} levels while observing changes in quantal release. The ionophore might also be used to transfer other divalent metal ions into the terminal, to see how effective they are compared with Ca^{2+} .

Using conventional methods, e.p.p.s and m.e.p.p.s were recorded intracellularly from the sartorius and cutaneous pectoris muscles of the frog *Rana pipiens*. Initial experiments were performed in our usual Ringer, based on an analysis of plasma¹¹, containing (mM): NaCl, 100; KCl, 2.0; CaCl_2 , 2.5; MgCl_2 , 3.0, and Tris-maleate buffer (pH 7.4), 8.0. In later experiments the Ringer was modified to contain only one metal chloride salt; in each case the concentration of the divalent ion is specified. All experiments were performed at 17° C. To record e.p.p.s, 3×10^{-6} g ml⁻¹ *d*-tubocurarine

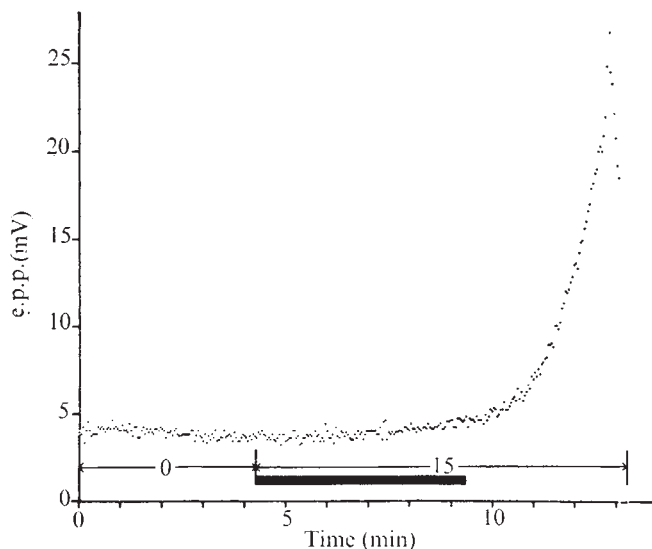


Fig. 1 The effect of X-537A on e.p.p. amplitude in a sartorius muscle. The sciatic nerve was stimulated supramaximally once every 2.5 s. The shaded region indicates the period during which the solution was being changed at a rate of 10 ml min⁻¹. The volume of the bath was about 5 ml. The numbers show the concentration of X-537A in μM .

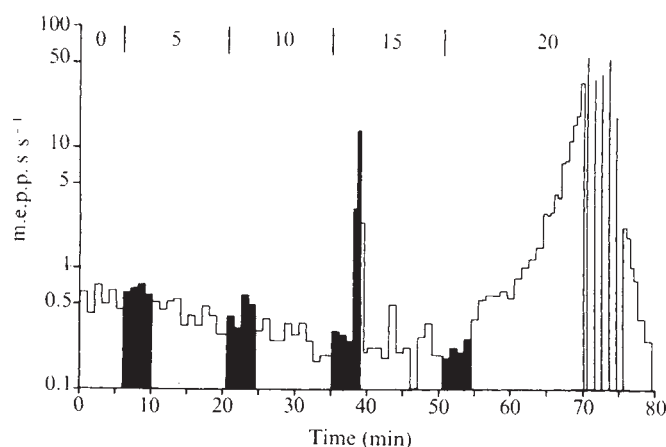


Fig. 2 The effect of a series of increasing concentrations of X-537A on the frequency of m.e.p.p.s. recorded from the sartorius muscle in 2.5 mM Ca^{2+} -Ringer. The width of each bar represents the period during which m.e.p.p. count was made. The shaded regions indicate the periods during which a solution change took place. The figures across the top show the X-537A concentration.

chloride was added to the Ringer. To increase the amplitude of m.e.p.p.s 1×10^{-6} g ml^{-1} neostigmine bromide was added.

The effect of X-537A on e.p.p. amplitude in 2.5 mM Ca^{2+} -Ringer is shown in Fig. 1. In Ringer the mean amplitude was 3.9 ± 0.3 mV (s.d., $n=103$). As Ringer containing 15 μM X-537A flowed into the chamber, the amplitude of the e.p.p. began gradually to increase. Within 205 s of the completion of the solution change, it reached a maximum of 26.9 mV. Then it declined precipitately and disappeared abruptly. In two other experiments similar responses were observed in 10 and 15 μM X-537A, though in these examples it took somewhat longer to reach a maximum e.p.p. and the increase was less marked. (10 μM X-537A: control 1.6 ± 0.4 mV ($n=101$) to a maximum of 5.5 mV. 15 μM X-537A: 1.1 ± 0.3 mV ($n=102$) to a maximum of 4.4 mV). Again the e.p.p.s disappeared abruptly within 4 min of reaching the maximum amplitude. Similar results were obtained from preparations in which transmission was blocked by increasing the Mg^{2+} concentration to 20 mM. The X-537A does not cause an increase in m.e.p.p. amplitude; in fact it may produce a decrease. For example, the mean m.e.p.p. amplitude in 2.5 mM Ca^{2+} -Ringer was 2.5 ± 0.85 mV (s.d., $n=50$; corrected to a standard membrane potential of 90 mV¹³). After 3 min in 15 μM X-537A the m.e.p.p. amplitude was 1.4 ± 0.35 mV ($n=50$). Another experiment gave a similar result. This means that the increase in e.p.p. amplitude produced by the drug must be caused by an increased release of acetylcholine from the motor nerve.

The effects of increasingly larger concentrations of X-537A on the frequency of m.e.p.p.s recorded in 2.5 mM Ca^{2+} -Ringer are shown in Fig. 2. X-537A at 5 or 10 μM did not elicit a change. During exposure to 15 μM there was a brief acceleration of m.e.p.p. frequency, lasting only about 90 s. With 20 μM there was a massive increase, followed by a decline to extremely low levels. In 2.5 mM Ca^{2+} , 3.0 mM Mg^{2+} -Ringer, 10 μM X-537A produced a notable increase in four of seven trials; 20 μM produced a notable increase in five of seven experiments. It is not uncommon for concentrations lower than those required for massive release to elicit a slight, transitory increase in m.e.p.p. frequency.

Figure 3 shows that the addition of X-537A to a Ringer solution containing no divalent cation does not change the m.e.p.p. frequency. The subsequent addition of 2.5 mM Ca^{2+} leads to a marked, transitory increase in m.e.p.p. rate. (In control experiments, the addition of 2.5 mM Ca^{2+} to a preparation in Ca^{2+} -free Ringer leads to about a two-fold, sus-

tained increase in m.e.p.p. frequency.)

Calcium is not the only effective metal. For example, the addition of 1.5 μM X-537A to a preparation soaked in 2.5 mM Ba^{2+} -Ringer led to a transitory increase in m.e.p.p. frequency which lasted for 55 min, the maximum frequency was 128 times the control level.

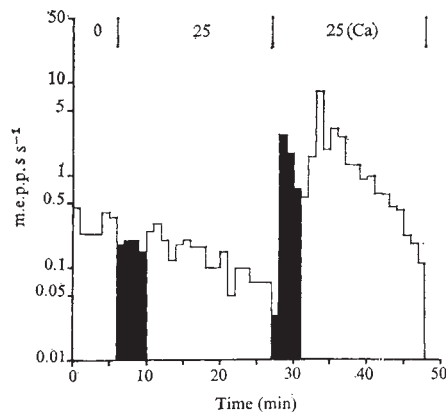


Fig. 3 The effects of Ca^{2+} on the response to X-537A. The cutaneous pectoris muscle was first soaked for 24 h in the refrigerator in a Ringer made without divalent cations but containing 1.0 mM MgEGTA. Then, as shown in the figure, the muscle was soaked in the same Ringer containing 25 μM X-537A during the period indicated as 25. The solution contained 25 μM X-537A and 2.5 mM Ca^{2+} but no MgEGTA during the period indicated as 25 (Ca).

A rough estimate of the relative effectiveness of different metals in accelerating spontaneous quantal release in the presence of X-537A can be drawn from Fig. 4. The apparent sequence is: $\text{Ba}^{2+} > \text{Sr}^{2+} > \text{Ca}^{2+} > \text{Mn}^{2+} \approx \text{Co}^{2+} \approx \text{Ni}^{2+} > \text{Mg}^{2+}$. For those metals for which both physiological and chemical data are available, the effectiveness in promoting spontaneous quantal release seems to parallel the ability of X-537A to carry the metal into a hydrophobic layer⁶.

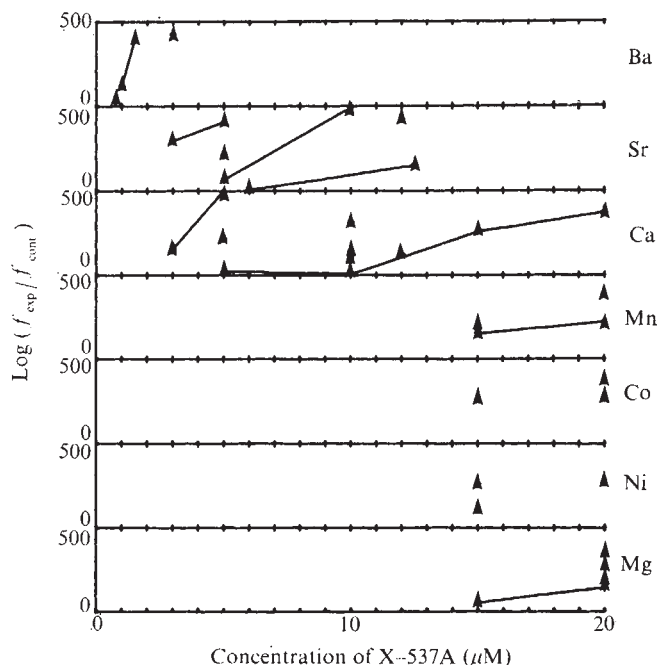


Fig. 4 The relative effectiveness of different metals in increasing m.e.p.p. frequency in the presence of X-537A. In each experiment, the muscle was first soaked in Ringer containing 2.5 mM of the indicated metal. The m.e.p.p. frequency in this solution is (f_{cont}). The maximum m.e.p.p. frequency in the presence of the indicated concentration of X-537A is (f_{exp}). The muscles were usually soaked for two hours in the metal Ringer before observations were begun. Values obtained from the same endplate are connected by lines.

When the preparation is exposed to effective concentrations of X-537A and a metal, the period of enhanced m.e.p.p. frequency lasts for 5–55 min. After the period of rapid release: (1) m.e.p.p.s are rarely seen; (2) in Ca^{2+} -Ringer, nerve stimulation does not elicit an e.p.p.; (3) tetanic nerve stimulation does not produce the usual increase in m.e.p.p. frequency; (4) a second application of X-537A does not produce a second increase in m.e.p.p. frequency. The reasons for the disappearance of m.e.p.p.s and for the abrupt failure of transmission are still uncertain. The compound action potential in the sciatic nerve is not affected by 25 μM X-537A, but we have not yet tested conduction in the terminals immediately above the end-plate.

In conclusion the Ca^{2+} -transporting antibiotic, X-537A, together with a divalent metal ion can elicit a substantial, transitory increase in m.e.p.p. frequency and in acetylcholine release elicited by nerve stimulation. The steep dose-effect curve suggests that the relation between intracellular metal concentration and rate of release is not linear. This finding may bear on the well known nonlinear relation between $(\text{Ca}^{2+})_0$ and quantal release from stimulated terminals^{13–15}. Once brought into the terminal by the ionophore, or by nerve stimulation¹⁶ each of several divalent metals increases the frequency of quantal release.

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Arginase affects lactogenesis through its influence on the biosynthesis of spermidine

THE activity of arginase in mammary gland increases markedly during lactation^{1–3}. Since lactating mammary gland, unlike liver, lacks other enzymes of the urea cycle³, the function of arginase in this gland is somewhat restricted. Results of earlier studies^{3,4} have suggested that the enzyme is involved in proline formation, in concert with ornithine aminotransferase, for increased synthesis of milk protein.

Another possible role for arginase is participation in the biosynthesis of spermidine. This possibility appears particularly attractive because the concentration of spermidine increases markedly in lactating tissue, as do the activities of

ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase⁵. In the presence of the latter two enzymes, ornithine formed by arginase can be converted to spermidine through putrescine⁶. Moreover, recent studies⁷ on the development of mammary gland *in vitro* have demonstrated that spermidine, together with insulin and prolactin, produces a marked increase in the synthesis of milk proteins, which is similar to that produced by the combination of insulin, glucocorticoid and prolactin. The involvement of spermidine in milk protein formation is further supported by the observation⁷ that the combination of these three hormones enhances the cellular concentration of spermidine before the accelerated synthesis of the milk proteins, casein and α -lactalbumin.

We have investigated the mechanism whereby the interplay of the hormones stimulates spermidine formation during mammary development. The data demonstrate that prolactin, in the presence of insulin, increases the activity of arginase in mammary epithelium. It appears that arginase may have a crucial function in milk protein synthesis by participating in the biosynthesis of spermidine.

The hormonal regulation of arginase activity was examined by culturing mouse mammary explants in a chemically defined medium containing several combinations of insulin, hydrocortisone and prolactin (Table 1). These hormones effect morphological and biochemical changes associated with mammary gland development⁸. Although mammary gland is primarily composed of epithelial cells and a large mass of fat cells during pregnancy, the activity of arginase in the gland was almost completely recovered in the homogenate of isolated epithelial cells⁹. After 3 d of culture, the initial level of arginase activity was maintained in the presence of insulin. Hydrocortisone by itself, or prolactin alone, or the combination of the two could not effectively maintain the initial level, and the enzyme activity decreased markedly as it did in the absence of any added hormones. In the presence of insulin, prolactin produced a

Table 1 Effect of various combinations of insulin, hydrocortisone, and prolactin on the activity of arginase in mammary explants in culture

Culture conditions	Arginase activity (μg urea formed per 10 min per mg wet wt tissue)
Single incubation	
Uncultured control	1.02 $\pm$ 0.05
No hormone	0.40 $\pm$ 0.08
Insulin	1.00 $\pm$ 0.07
Hydrocortisone	0.38 $\pm$ 0.04
Prolactin	0.41 $\pm$ 0.04
Hydrocortisone + prolactin	0.38 $\pm$ 0.05
Insulin + prolactin	3.20 $\pm$ 0.08*
Insulin + hydrocortisone	1.28 $\pm$ 0.04*
Double incubation	
Control	1.24 $\pm$ 0.08
Insulin	1.31 $\pm$ 0.05
Prolactin	0.47 $\pm$ 0.04
Insulin + hydrocortisone	1.40 $\pm$ 0.09
Insulin + prolactin	3.10 $\pm$ 0.08*
Insulin + hydrocortisone + prolactin	4.43 $\pm$ 0.07*

Mammary explants from midpregnant C3H/HeN mice were cultured in Medium 199 (Gibco) containing the indicated combination of hormones as described previously¹⁰. The final concentration of hormones was 5 μg ml⁻¹ for insulin and prolactin, and 0.5 μg ml⁻¹ for hydrocortisone. Single incubation was carried out for 72 h. Double incubation was carried out by first culturing mammary explants in insulin-containing medium for 72 h and then transferring explants to medium containing the indicated combination of hormones. After transfer the explants were cultured for another 72 h. Control in the double incubation system refers to explants cultured with insulin for the initial 72 h.

The activity of arginase was determined by measuring the formation of urea from arginine as described earlier¹¹. Each value represents the mean $\pm$ s.e.m. of six separate determinations on three separate cultures.

* The increase is significant over control ($P < 0.01$).

Table 2 Effect of various culture conditions on α -lactalbumin activity in mammary gland explants

Culture conditions	α -Lactalbumin activity (pmol lactose formed per 30 min per mg wet wt tissue)
Uncultured control	20 $\pm$ 5
Three hormones in regular M199	90 $\pm$ 7
Three hormones in arginine-deficient M199	32 $\pm$ 4
Three hormones + L-ornithine in arginine-deficient M199	95 $\pm$ 6
Three hormones + putrescine in arginine-deficient M199	91 $\pm$ 6
Three hormones + spermidine in arginine-deficient M199	85 $\pm$ 5
Three hormones + proline in arginine-deficient M199	30 $\pm$ 5
Three hormones + lysine in arginine-deficient M199	22 $\pm$ 4
Three hormones + spermine in arginine-deficient M199	10 $\pm$ 4

Mammary explants from midpregnant C3H/HeN mice were cultured for 48 h in the presence of insulin, hydrocortisone and human placental lactogen. The final hormone concentration was 5 μ g ml⁻¹ for insulin and hydrocortisone, and 1 μ g ml⁻¹ for human placental lactogen. Human placental lactogen was previously shown to be as effective as prolactin in stimulating milk protein synthesis in mammary explants¹⁹. Arginine-deficient Medium 199 contained 0.2 mM L-arginine, whereas regular Medium 199 contained 4 mM L-arginine. The final concentration of other added amino acids was 0.05 mM L-ornithine, 4 mM L-proline, and 5 mM L-lysine. The final concentration of putrescine, spermidine and spermine was 4 mM.

The activity of α -lactalbumin was determined by measuring the formation of lactose from uridine-diphospho-¹⁴C-galactose (290–300 mCi mmol⁻¹, New England Nuclear Corp.) and glucose as described previously¹⁹. Each value represents the mean $\pm$ s.e.m. of four separate determinations on two cultures.

large increase in the enzyme activity, whereas hydrocortisone consistently produced a slight increase.

Insulin stimulates cell proliferation in mammary epithelium during the first 2 d of culture, but by the third to fourth day the proliferative activity disappears¹⁰. These non-proliferative cells are still viable since they synthesise milk proteins in response to insulin, glucocorticoid and prolactin^{11,13}. Table 1 shows that addition of prolactin to the culture of such cells also resulted in increased arginase activity. The stimulatory effect of prolactin was enhanced by hydrocortisone although the steroid alone, or in combination with insulin, produced little increase in arginase activity in post-mitotic cells. It is clear that the effect of prolactin again required the presence of insulin, since no stimulation occurred when insulin was omitted during the second incubation. These results indicate that stimulation of arginase activity by prolactin is not a consequence of the increase in the number of mammary epithelial cells since proliferation of mammary epithelium was essentially completed when prolactin was added.

We next assessed the possibility that arginase participates in milk protein synthesis by supplying ornithine for the formation of spermidine (Table 2). When mammary explants were cultured in medium in which the concentration of arginine was reduced from 4 mM to 0.2 mM, the triple hormone combination produced very little increase in the synthesis of α -lactalbumin. However, addition of ornithine, the product formed by arginase, to such a medium restored the increase in α -lactalbumin. Addition of the polyamines, putrescine and spermidine, was also effective, whereas another polyamine, spermine, and amino acids such as proline and lysine did not effect an increase in α -lactalbumin. The effect of arginine deprivation is specific in the sense that in experiments where the concentration of other amino acids, such as leucine, was similarly reduced in the culture medium the three hormones produced an increase in α -lactalbumin similar to that observed in the regular medium 199 (not shown). These results can best be explained by the possibility that arginine may be converted, through ornithine and putrescine, to spermidine, which is required for milk protein synthesis^{7,14}. Preliminary studies indicate that ³H-arginine is indeed converted to ³H-spermidine and that prolactin stimulates this conversion by enhancing the activity of arginase (our unpublished

results). These results strongly suggest that arginase plays an important role in the biosynthesis of spermidine. Earlier studies^{14,15} on cultured mammary explants showed that insulin stimulates the activity of ornithine decarboxylase and that glucocorticoid increases the activity of S-adenosyl-L-methionine decarboxylase. Thus it appears that insulin, glucocorticoid, and prolactin interact to stimulate a group of enzymes which are involved in the biosynthesis of spermidine during the development of mammary epithelium. In view of the observation^{7,14} that spermidine is necessary for milk protein synthesis, such interaction may represent a fundamental mechanism for the regulation of lactogenesis by these hormones.

Finally, it should be emphasised that our studies *in vitro* do not exclude a possible role of arginase for proline formation, as suggested earlier³. It is possible that in addition to a role in spermidine formation, arginase may also participate in proline synthesis in intact animals where the proline supply may not be abundant.

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Cytosol-binding protein of thyroxine and triiodothyronine in human and rat kidney tissue

DURING the past few years, it has become increasingly apparent that nonpeptide hormone action in the respective target organs occurs in the nucleus. This was first shown for steroid hormones^{1–4} and more recently for thyroid hormones⁵. Specific nuclear acceptor molecules for hormones have been shown in target tissues, associated with nuclear proteins^{6–8}. The question arises whether hormone molecules, on their way to the nuclear binding sites, traverse the cytoplasm in a more or less random fashion or whether they are bound by specific cytoplasmic receptor molecules.

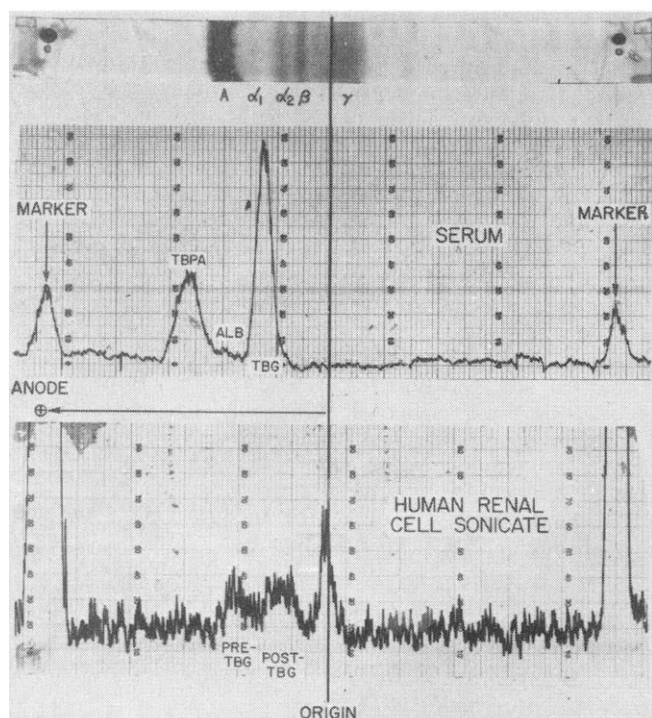


Fig. 1 Comparison of radioactive scans of human serum and human renal cell binding proteins. Electrophoresis was done in glycine-acetate, pH 8.6. The label is $^{125}\text{I}-\text{T}_4$.

Accordingly we have sought specific cytoplasmic proteins that bind thyroid hormones and are distinctly different from the serum protein carriers such as thyroxine-binding α -globulin (TBG), thyroxine-binding prealbumin (TBPA) and albumin, the major carriers of thyroid hormones in the circulations of rats as well as humans. In particle-free cytosol preparations from rat kidneys we have found four separate protein peaks, three of which bind both thyroxine (T_4) and triiodothyronine (T_3). One peak seems to bind T_3 only. Thus, the cytoplasmic transport of thyroid hormones seems to depend on receptor molecules.

Male Sprague-Dawley rats weighing in excess of 250 g were used. Cytosol was prepared from perfused rat kidneys and livers. The aorta was cannulated and the kidneys perfused *in situ* for 20–30 min with ice-cold isotonic saline solution until the venous effluent was crystal clear. Subsequent protein determinations by the Oyama-Eagle modification⁹ of the Lowry method¹⁰ confirmed the absence of appreciable protein in the effluent irrigation solution. The kidneys were removed and homogenised in three volumes of saline solution in an all glass homogeniser. In some cases the homogenate was spun at 800g to obtain a supernatant free of nuclei and gross membrane fragments. The supernatant or the homogenate, packed in ice, was sonicated for 3 min with a Savant Instrument Model 1000 Insonator. The sonicated material was centrifuged for 1 h at 105,000g in a Beckman L3-50 ultracentrifuge. The temperature throughout this procedure was 0°–4° C. After centrifugation the supernatant was decanted and stored at –20° C until used.

The cytosol proteins were labelled with $^{125}\text{I}-\text{T}_3$ or with $^{131}\text{I}-\text{T}_4$ (Industrial Nuclear) and subjected to paper electrophoresis. The electrophoretic systems were 0.2 M glycine-acetate, pH 8.6 (refs 11, 12), 0.125 M sodium borate buffer, pH 10.0, or 0.05 M sodium glycinate buffer, pH 10.0. The paper strips were dried after electrophoresis and scanned with a Nuclear-Chicago Actigraph III (Model 1004) strip scanner. In case of dual labelling, the strips were scanned rapidly and the area containing radioactivity was cut into 0.5 cm segments, which were then counted in a Packard Auto-Gamma spectrometer. Cultures of human kidney and liver cells were purchased from Microbiological Associates and treated as described earlier¹³.

The total protein content of the effluent perfusate was less than 2 mg ml⁻¹ after 20 ml of effluent had been collected, and less than 1 mg ml⁻¹ after 150 ml had been collected, which usually required 20–30 min of perfusion. The liver perfused through the portal vein exhibited blanching, but effluent fluid was not analysed.

It was not unexpected therefore, that cytosol preparations subjected to electrophoresis showed no peak with the mobility of serum TBG. In all cases, cytosol-binding proteins (CBP) were readily evident on scanning of dried paper electrophoretic strips; the scanning was done before staining with bromphenol blue to avoid elution of the tracer.

The dialysis of the cytosol against at least 100 volumes of buffer or saline solution overnight before electrophoresis revealed retention of more than 60% of the radioactivity by the cytosol solution in the dialysis bag, signifying unequivocal protein binding.

Gel filtration studies on Sephadex G-200 showed a protein peak of radioactivity compatible with a molecular weight of about 70,000. To investigate the existence of cytosol binders in human kidney tissue, similar electrophoretic studies were carried out on sonicated human renal cells which had been incubated with $^{125}\text{I}-\text{T}_4$ as previously reported¹³. A typical scan is illustrated in Fig. 1, which shows 'pre-TBG' and 'post-TBG' peaks, so designated because of their mobility, as well as origin radioactivity. The low level of radioactivity in human renal cell sonicates reflected the tracer amounts of hormone added to the cell cultures, approximately 2×10^{-8} M T_4 .

The amount of origin radioactivity in different human and rat cell studies was quite variable, occasionally absent, and not clearly associated with any obvious experimental detail.

Experiments were also performed on perfused rat liver cytosol as well as human liver cells (Chang liver) grown in culture¹³, with findings of cytosol binding proteins in all cases.

As Fig. 2 shows, labelled T_3 was apparently bound by different cytosol-binding proteins than T_4 , as illustrated in electrophoretic strips run simultaneously.

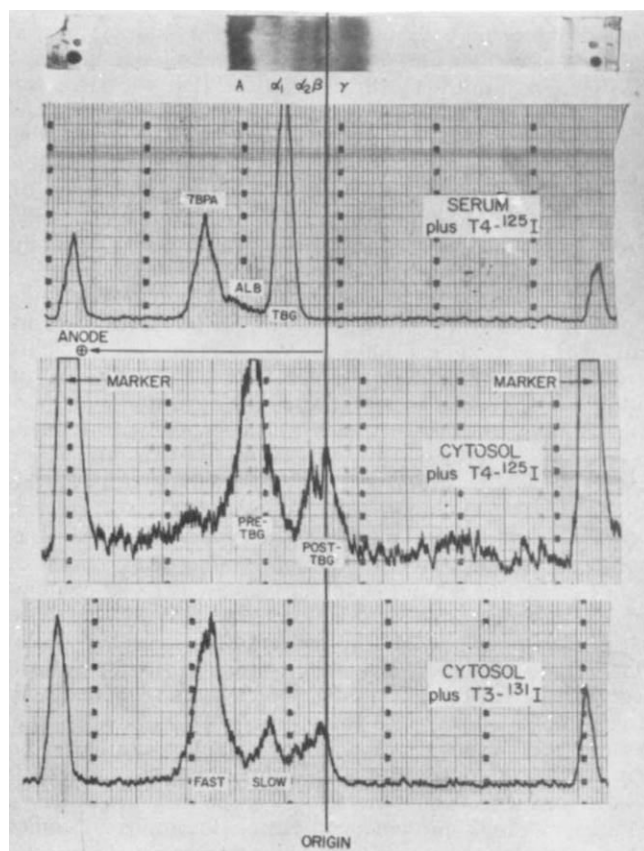


Fig. 2. Rat renal cytosol binding proteins for T_4 and T_3 . Electrophoresis was done in glycine-acetate, pH 8.6.

Since it could be argued that the different ligands could conceivably cause slightly different electrophoretic mobilities, experiments were carried out in which both labelled hormones as ^{125}I - T_3 and ^{131}I - T_4 were added to the same rat renal cytosol preparation. A peak with greatest anionic mobility was found to bind T_3 but not T_4 , even with increments of nonradioactive T_4 as high as 20 mg per 100 ml (2.6×10^{-7} M) as illustrated in Fig. 3. Whereas the glycine-acetate system at pH 8.6 used in the foregoing studies provided reasonable separation of peaks, we found that the sodium borate and sodium glycinate buffer systems at pH 10.0 provided much more rapid migration and greater separation of cytosol binding proteins (although these alkaline buffers are less satisfactory for study of serum protein carriers).

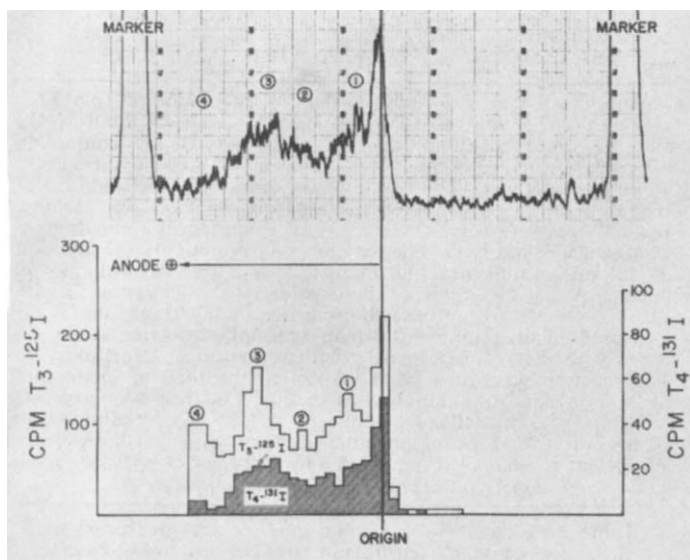


Fig. 3 Rat renal cytosol binding proteins for T_4 and T_3 . Cytosol contained both ^{125}I - T_3 and ^{131}I - T_4 . Electrophoresis was done in sodium glycinate buffer, pH 10.0. The radioactive scan above reflects mainly ^{131}I , and ^{125}I to a lesser degree. In the histogram below with discrimination between the labels, in addition to origin radioactivity, the labelled T_3 is distributed in four peaks, appropriately numbered, with 4, which has moved farthest toward the anode at the left, clearly showing T_3 , but no appreciable T_4 radioactivity. In contrast, the other peaks bound both radioactive hormones. In the run illustrated, the concentration of T_3 added was 0.16 μg per 100 ml (2.4×10^{-8} M), while the T_4 was 11 μg per 100 ml (1.4×10^{-7} M). Similar results, that is, peak 4 showing T_3 but not T_4 radioactivity were also seen with the same T_3 concentration, but T_4 concentrations of 1.1, 6.1, and also 21.1 μg per 100 ml (1.4×10^{-8} M, 7.9×10^{-8} M and 2.7×10^{-7} M).

The basic finding is the demonstration of binding of thyroid hormones by cytosol with retention of the ligand hormones after overnight dialysis. The demonstration of electrophoretic peaks suggests that it may become possible to isolate specific cytosol binding proteins and determine their binding constants by equilibrium dialysis and other methods. The findings are in general agreement with the preliminary descriptions of cytosol binding proteins by others¹⁴⁻¹⁷.

The use of strongly alkaline (pH 10.0) buffers represents a novel technical approach, in that it enhanced the sensitivity of ordinary paper electrophoresis with conventional rather than 'reverse' or countercurrent flow¹⁸. Since the overall picture is not different from those runs with lower pH, it is reasonable to assume that the only effect of the high pH was increased separation.

Hormone action at the molecular level seems to involve the interaction between the hormone and the nonhistone or acid soluble protein associated with nuclear chromatin, when small molecule (nonpeptide) hormones are considered. The evidence amassed during the past decade includes more evidence for the

action of the steroid hormones than for the thyroid hormones. Thus numerous studies have dealt with the sex steroids^{19,20} cortisol²¹ and aldosterone^{22,23}. The thyroid hormones, on the other hand, have been considered to have a primary action on the cell nucleus since the demonstration of nuclear localisation of labelled thyroid hormone by Siegal and Tobias²⁴ in cultured human renal epithelial cells. Further evidence concerning nuclear binding of the thyroid hormones has been found in our laboratory, in which nuclear uptake *in vitro* has been shown, and further details have been reported by others⁵⁻⁷. Since there is also evidence of mitochondrial mediation of thyroid hormone action²⁵⁻²⁸, it is interesting that we have found mitochondrial protein binding of both T_4 and T_3 , in studies now in progress.

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Aryl hydrocarbon (benzo(a)pyrene) hydroxylase in human peripheral blood monocytes

MICROSOMAL mixed-function oxygenases metabolise a variety of exogenous compounds, including drugs, pesticides and carcinogens, as well as endogenous substances such as steroids^{1,2}. One oxygenase, aryl hydrocarbon hydroxylase (AHH), is important in both the detoxification of some carcinogenic polycyclic hydrocarbons and the activation of others to more carcinogenic forms¹⁻⁴. Hydroxylase activity and cytochrome P-450 have been found in various tissues of animals^{1,2} and man⁵⁻¹². AHH activity increases in many tissues after treatment with polycyclic hydrocarbons. In certain inbred mouse strains this increase appears to be regulated by a single autosomal dominant gene¹³, and a correlation between enzyme levels and susceptibility to methylcholanthrene-induced tumorigenesis has been reported¹⁴. Data obtained by Shaw and his colleagues point to genetic regulation of AHH in human populations¹⁵.

With the exception of peripheral blood lymphocytes, which must be stimulated with mitogens before assay, none of the human tissues in which AHH has been detected can be obtained readily for clinical and epidemiological study. Detection of AHH in macrophages of the human, lung¹⁰, rat liver¹⁶ and guinea pig peritoneum¹⁷ suggested that monocytes might also contain the enzyme. We report here that AHH is present in monocytes from human peripheral blood. This observation may facilitate studies in human populations relating hydroxylase activity and the metabolism of chemical carcinogens.

Mononuclear leukocytes were obtained from heparinised human blood by the method of Boyum¹⁸. At least 99% of the cells isolated were mononuclear and excluded trypan blue. After incubation in cell culture for 24 h, the leukocytes were treated with medium that either contained or lacked the polycyclic hydrocarbon benz(a)anthracene (1 $\mu\text{g ml}^{-1}$). After incubation for a further 17 h, the culture medium was removed and the surface of the plastic culture dish was rinsed with Dulbecco's phosphate-buffered saline. The adherent cell population was more than 95% viable and contained 98% monocytes, judged by cellular morphology and by the ability of the cells to phagocytose 1 μm latex spheres (Dow) suspended in Eagle's minimum essential medium containing 10% pooled human AB serum. An average of 46×10^6 monocytes was obtained from 500 ml of heparinised blood. Treatment with benz(a)anthracene did not affect the yield, viability or differential cell count of these preparations. Monocytes were assayed for AHH activity by the method of Nebert and Gelboin¹⁹ as modified by Kellerman *et al.*¹⁵.

AHH is present in human peripheral blood monocytes and its activity is increased following treatment with benz(a)anthracene (Table 1). In twelve different donors, AHH activity ranged from 0.3 to 1.4 units per 10^6 cells and increased 4 to 33-fold after incubation with benz(a)anthracene. The activity of replicate samples from a single monocyte pool varied by less than 5%. AHH from human peripheral blood monocytes has properties which are typical of the microsomal mixed-function oxygenases (Table 2), that is, a requirement for NADPH and inhibition by carbon monoxide^{11,18}. In addition, hydroxylase activity is inhibited by 7, 8-benzoflavone, a potent inhibitor of the complex²⁰.

The presence of AHH in human peripheral blood monocytes further implicates the reticuloendothelial system in the metabolism of polycyclic hydrocarbons, suggests one explanation for the immunogenicity of these compounds and may provide a convenient assay for the study of AHH in human populations. Peripheral blood monocytes contribute to macrophage pools throughout the body and accumulate at sites of acute and chronic inflammation²¹. Circulating monocytes could provide large local concentrations of the enzyme at inflammatory foci. Cantrell *et al.*¹⁰ obtained

larger numbers of alveolar macrophages with higher hydroxylase activity per cell from the lungs of smokers than from those of nonsmokers. The infiltration of inflammatory cells evoked by cigarette smoke, by various carcinogens and by cocarcinogenic promoters might influence the local metabolism of polycyclic hydrocarbons. Reticuloendothelial cells in the liver, lung and lymphoid tissue might also participate in the detoxification or activation of certain carcinogens. Agents that stimulate or depress the reticuloendothelial system, such as BCG can modify chemical carcinogenesis²². The functional state of the reticuloendothelial system might alter the metabolic fate of carcinogenic compounds.

Table 1 Aryl hydrocarbon hydroxylase activity in human peripheral blood monocytes

	Specific activity*	
	Donor 1	Donor 2
Control	0.5	1.4
BA-treated	12.9	19.3

Monocytes were suspended in 0.85 ml of a solution (pH 7.55) containing 67.5 $\mu\text{mol KH}_2\text{PO}_4$, 66.7 $\mu\text{mol KOH}$, 4.2 $\mu\text{mol MgCl}_2$ and 25 μmol nicotinamide. A solution (100 μl) containing 0.7 mg NADH, 0.7 mg NADPH and 0.7 mg bovine serum albumin was added. The reaction was started by the addition of 100 nmol of the substrate benzo(a)pyrene, in 0.050 ml acetone. Incubations were for 20 min at 37°C under the illumination of a single 25 W red bulb. The reaction was stopped by the addition of 1.0 ml acetone and the mixture was extracted with 3.0 ml hexane. A 2.5 ml sample of the organic layer was extracted with 1.0 ml 1 N NaOH, and the fluorescence of the alkali phase was measured in an Aminco-Bowman spectrofluorometer and compared with that of a standard 3-hydroxybenzo(a)pyrene solution. The reaction was linear with respect to the time of incubation (0-20 min) and the number of monocytes that was assayed.

*Units per 10^6 cells. One unit of AHH activity catalyses in 20 min the formation of phenolic products with the fluorescence equivalent to that of 1 pmol of 3-hydroxybenzo(a)pyrene. Each assay contained $1.4-3.3 \times 10^6$ monocytes

Table 2 Effect of assay conditions on aryl hydrocarbon hydroxylase activity from human peripheral blood monocytes

Assay system	Specific activity*
Complete	5.0
No NADPH	1.5
90% CO-10% O ₂	1.6
7,8 BF (10^{-4}M)†	2.0

Monocytes were treated with benz(a)anthracene (1 $\mu\text{g ml}^{-1}$) as outlined in the text. AHH was assayed as described in Table 1. *Units per 10^6 cells. Each assay contained 3.3×10^6 monocytes.

†7,8 benzoflavone was added in 0.010 ml dimethylsulphoxide.

Cutaneous application of polycyclic hydrocarbon carcinogens can produce immunologically specific contact hypersensitivity²³. These low molecular weight hydrocarbons probably attain immunogenicity by conjugation with host proteins. Microsomal enzymes might contribute to the formation of immunogenic conjugates by converting unreactive parent compounds to reactive intermediates able to bind covalently to cellular macromolecules. Macrophages derived from peripheral blood monocytes process macromolecular antigens for presentation to lymphocytes in a more immunogenic form²⁴. AHH within macrophages could catalyse the formation of immunogenic conjugates before antigen processing.

A recent study has attempted to relate AHH levels to the development of lung cancer in man. Kellerman *et al.*²⁵ have measured hydroxylase induction in human leukocytes after incubation with phytohaemagglutinin and pokeweed mitogen. Under these conditions the polycyclic hydrocarbon 3-methylcholanthrene induced a greater increase in hydroxylase activity in leukocytes from patients with lung cancer than in leukocytes from a control population. The requirement for mitogens in this system, however, raises the possibility that hydroxylase levels might be a function of the response of leukocytes to the mitogens rather than their response to the 3-methylcholanthrene. Treatment with mitogens is not required to measure AHH in monocytes

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ALMOST 30 years after the initial discovery of the prostaglandins, Bergstrom *et al.*, succeeded in isolating and identifying prostaglandins (PGs) E₁, E₂, F_{1α} and F_{2α} from sources including human semen¹. It was subsequently claimed² that the 19-hydroxy derivatives of prostaglandins A and B were also present. These compounds were later found at an average

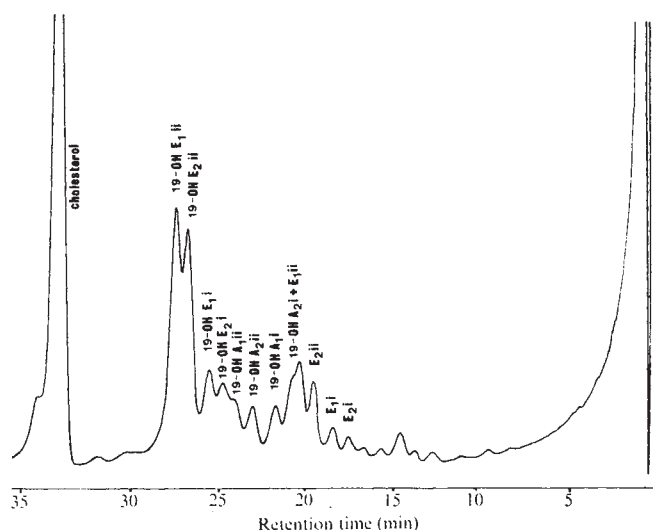


Fig. 1 Gas chromatogram of a semen extract as methyl oximes, methyl esters and trimethyl silyl ethers. The extract was prepared by mixing the semen with four volumes of 1.5 M pyridinium acetate buffer (pH5) containing 5 mg ml⁻¹ methoxyamine hydrochloride and leaving in an ultrasonic bath for 40 min. The prostaglandins were then extracted with ether/ethyl acetate (4:1), methylated (diazomethane) and silylated (*bis*-(trimethylsilyl) acetamide). The oximation gives rise to *syn* and *anti* isomers; in the E compounds the first isomer to elute accounts for approximately 1/4 of the material derivatised⁵. The isomers are denoted by suffixes (in roman numerals) giving the order of elution. Identification of the components was by gas chromatography/mass spectrometry. The column was 1% of Dexsil 300 on chromosorb G and was programmed from 200° to 2° min⁻¹.

concentration of 40 $\mu\text{g ml}^{-1}$ (ref. 3). In a detailed study of semen PGs we have used a new approach for the measurement of the E prostaglandins, which involves protecting the unstable β -ketol system by oximation in the unextracted semen, thus ensuring minimal degradation to the A or B series. Using this technique we found that the levels of 19-hydroxy derivatives of PGA and PGB are relatively low whereas two new PGs, later identified as 11 α ,15,19-trihydroxy-9-keto prost-13-enoic acid (19-hydroxy PGE₁) and 11 α ,15,19-trihydroxy-9-keto prosta-5,13-dienoic acid (19-hydroxy PGE₂) were present at an average total concentration of 100 $\mu\text{g ml}^{-1}$. These compounds can be isolated together as the methyl ester/methyl oxime derivatives by thin layer chromatography and the individual components can be separated by gas chromatography as the methyl ester/methyl oxime/*tert*-butyl dimethyl silyl ethers⁴.

The nature of the 19-hydroxy prostaglandins E was determined by gas chromatography/mass spectrometry and thin layer chromatography. The compounds were present in gas

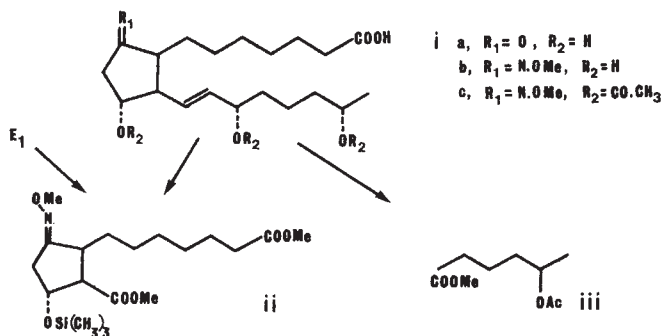


Fig. 2 Oxidative degradation of 19-OH PGE₂ as proof of structure. **ia** is 19-OH PGE₂, periodate/permanganate oxidation of the derivatised **1b** followed by methylation (diazomethane) and silylation (*bis*-(trimethylsilyl) acetamide) gives **ii**. Oxidation of **ic** followed by methylation gives **iii**. Compound **ii** was also obtained by a similar oxidation of authentic PGE₂.

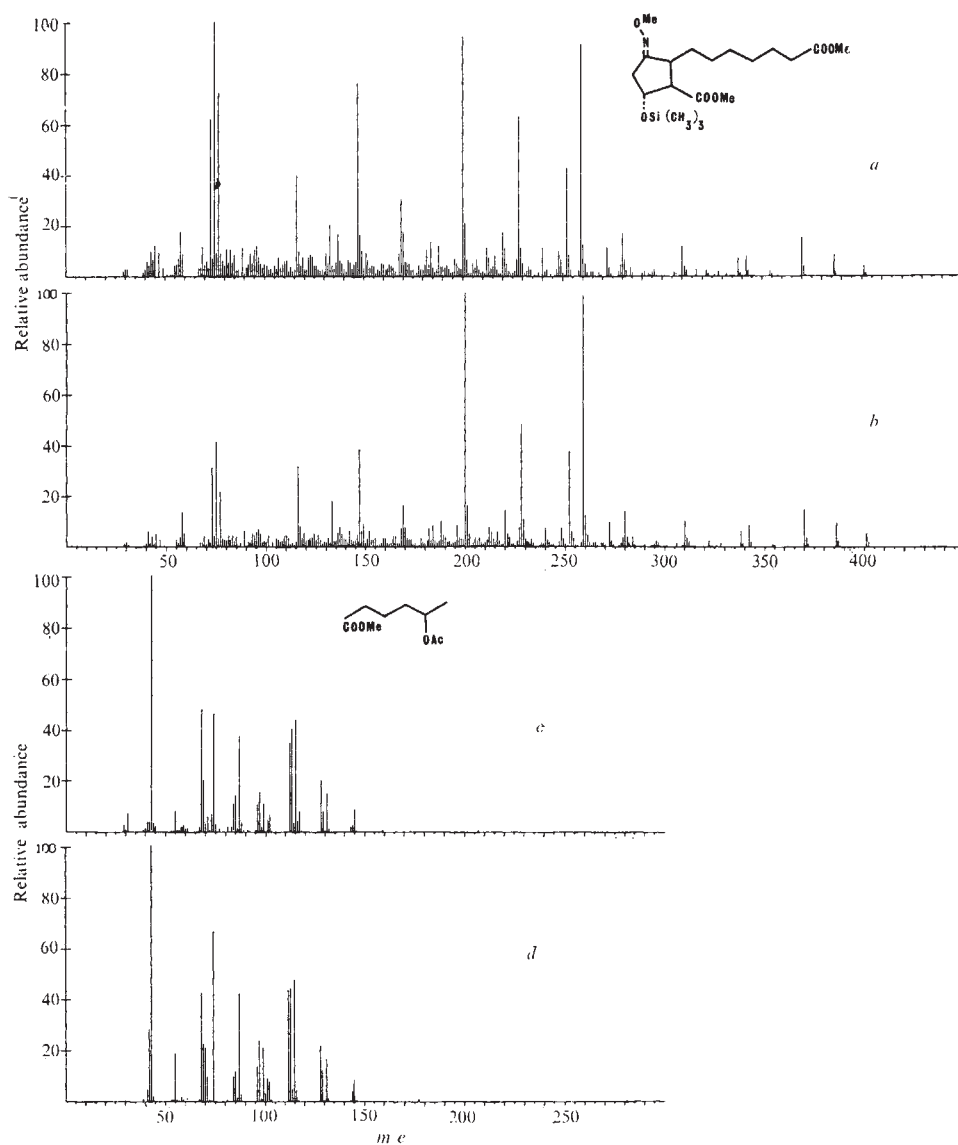


Fig. 3 Mass spectra of oxidation products of 19-OH PGE₁ (*a* and *c*) and spectra of authentic origin; (*b*) was obtained by the oxidation of PGE₁, and (*d*) was prepared by methylation, borohydride reduction in tetrahydrofuran and acetylation of 5-keto hexanoic acid.

chromatograms of semen extracts as a pair of peaks, (19-OH E₂) followed by (19-OH E₁), of approximately equal size, having retention times longer than those of E and 19-OH A prostaglandins (Fig. 1). The mass spectral evidence (Table 1) was wholly consistent with the assigned structures. The shift of 14 mass units in the molecular ion between a methyl and an ethyl oxime indicated one carbonyl group and a shift of 126 between the molecular ion of the trimethyl silyl ether derivative and that of the *tert*-butyl dimethyl silyl derivative implied three hydroxyl groups. The mass spectra of all derivatives showed many similarities to those of the E prostaglandins; particularly informative was a strong M-159 peak in the spectrum of the methyl oxime/methyl ester/TMS ether repre-

senting the loss of C₁₆ to C₂₀ of the prostaglandin molecule. This ion was particularly strong in the first isomer of the pair arising from oxidation. The loss of 159 mass units from the 19-OH compound corresponds to the loss of 71 mass units from the parent E derivative. This ion is similarly strong in the first isomer of E to elute on gas chromatography⁵. The presence of an ion of *m/e* 117 in all mass spectra of the TMS ether derivative of the 19-hydroxy compounds supported the assignment of the extra hydroxyl group to the 19 position, as this ion is characteristic of the CH (OTMSi) CH₃ group.

The identity of the 19-OH E prostaglandins was confirmed by oxidative degradation. The 19-OH E compounds were isolated as a group by TLC using the methyl ester/methyl

Table 1 Prominent peaks in the mass spectra of the two isomers of 19-OH E₁s—19-OH E₂s as methyl oximes, methylesters, trimethyl silyl ethers

Ion <i>m/e</i>	19-OH prostaglandin E ₂		Probable origin	Ion <i>m/e</i>	19-OH prostaglandin E ₁		Probable origin
	First isomer Relative abundance (%)	Second isomer Relative abundance (%)			First isomer Relative abundance (%)	Second isomer Relative abundance (%)	
627		3	M	598	18	5	M-31
596	18		M-31	508	17	5	M-(90+31)
537		16	M-90	470	60	4	M-159
506	16	27	M-(90+31)	456		8	M-(C ₁ -C ₇)
468	20	7	M-159	418	17	6	M-(180+31)
416	12	8	M-(180+31)	380	45		M-(159+90)
378	25		M-(159+90)	366		74	456-90

Ninety mass units represents loss of (–OTMSi+H), 31 mass units represents loss of –OCH₃ from methyl oximes.

oxime derivatives. These derivatives were recovered from the TLC plate and were oxidised with periodate/permanganate as described by Jacobson *et al.*⁶. This reagent hydroxylates double bonds and cleaves adjacent oxygenated functions. Thus hydroxyl groups are introduced at C₁₃ and C₁₄ and cleavage occurs both between C₁₃ and C₁₄ and between C₁₄ and C₁₅ (Fig. 2). To identify the fragment C₁ to C₁₃ the products of the oxidation of 1b were methylated, silylated and analysed by gas chromatography mass spectrometry. The spectrum of the expected compound (II) correlated well with the same fragment obtained by a similar oxidation of authentic PGE₁ (Fig. 3). To identify the C₁₅–C₂₀ fragment the 19-OH prostaglandins E were first acetylated (to prevent lactone formation after oxidation) and then oxidised as before. Gas chromatography mass spectrometry analysis showed the major product to be 5-acetoxy methyl hexanoate, identified by comparison with the spectrum from authentic material (Fig. 3).

The identity of these two parts of the molecule together with the mass spectral information from the intact compound conclusively identify the material present in the second gas chromatography peak as 19-OH PGE₁. The similarity in the mass spectra and the gas chromatography evidence strongly indicate that the first peak of the pair is 19-OH PGE₂.

These 19-OH prostaglandins have probably been missed before for various reasons. They are converted to the A series if left in semen (almost all 19-OH prostaglandins E had disappeared in a semen sample left at 37° C for 60 h) and they are more difficult to extract from semen than the hydroxylated A or B compounds. We have succeeded in identifying the compounds by immediate oxidation of fresh semen samples followed by extraction. We are now using this technique to establish the range of levels of these compounds in normal males and in cases of infertility.

The biological role of prostaglandins in human semen has yet to be established but it is attractive to suppose that they could be concerned with sperm migration; it has even been claimed that PGE levels are lower in the semen of infertile men³. This leads us to pursue the investigation of the biological activity and physiological significance of these exciting compounds.

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identified and quantitated³, and the macrophage contents of different experimental tumours were found to range from 4%–56% of the total cell population³. These cells were shown to be of host origin, most if not all being derived from circulating blood monocytes rather than by self-replication of macrophages within the tumour (Unpublished observation).

The experiments described here were designed to investigate whether the macrophage contents of a group of chemically induced rat fibrosarcomata were in any way related to their biological behaviour, and in particular to their metastatic capacity. It is possible that the number of monocytes gaining entry into a tumour is governed solely by such properties as its vasculature, or alternatively, the presence of macrophages may represent part of an immunological

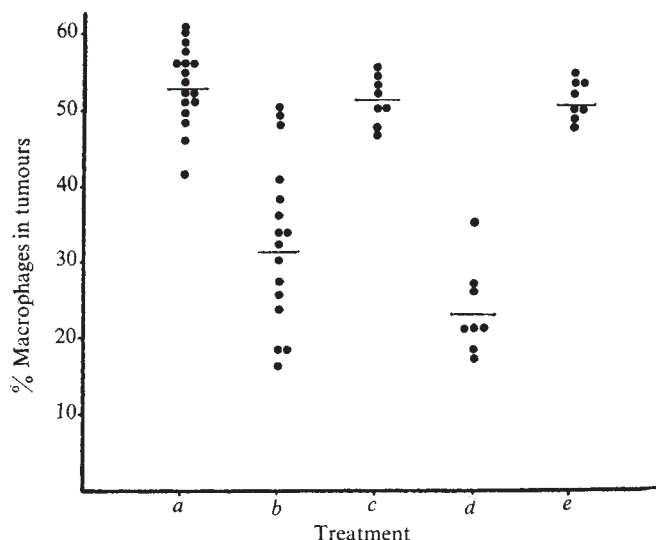


Fig. 1 Effect of immunosuppression on % macrophages in tumours grown in deprived rats. *a*, Controls; *b*, rats with thoracic duct lymph drainage; *c*, sham cannulated rats; *d*, thymectomised and irradiated rats; *e*, sham thymectomised rats. The thoracic ducts of rats were cannulated and tumours implanted 1 d later. Drainage was terminated after 8–10 d when the number of lymphocytes collected over 24 h had fallen to 0.1% of the first day's yield. Sham cannulated rats underwent anaesthesia and laparotomy, but the duct was left intact. Other rats were thymectomised at 4 weeks and then received 3 × 300 rad whole-body irradiation (X rays) at 2 week intervals. To ensure recovery of the bone marrow, an interval of 4 weeks was allowed between the last dose of irradiation and implantation of the tumour. At this time, treated rats were able to develop normal inflammatory macrophage exudates in response to intraperitoneal oyster glycogen stimulation⁶, indicating normal monocyte production. Thymuses of sham thymectomised rats were exposed but not removed and received no irradiation. The tumour used was a benzpyrene-induced fibrosarcoma (HSBPA) which had been passaged 20–25 times in syngeneic Hooded rats. All tumours were grown intramuscularly (i.m.) in hind limbs, excised 14 d later when they were approximately 2 cm diameter, and their macrophage contents assessed by Evans' methods³. Each point represents one rat, and the horizontal bar is the mean of the group.

Macrophage content of tumours in relation to metastatic spread and host immune reaction

THE presence of infiltrating 'histiocytic', 'mononuclear' or 'round' cells (many of which may be cells of the monocyte/macrophage series) has often been observed in histological sections of certain types of human tumours and has been claimed to indicate good prognosis^{1,2}. Evans, using a variety of functional criteria (such as adhesion to glass in the presence of trypsin and phagocytic ability) has shown that macrophages in tumour cell suspensions can be readily

reaction to tumour antigens and the degree of macrophage infiltration could then be a measure of the host response.

The macrophage content of tumours grown in immunosuppressed Hooded rats was monitored. For these experiments to be interpretable, it was necessary to suppress immunity without affecting normal bone marrow function, so that the availability of monocytes would not be reduced. The procedures chosen were two methods which removed predominantly T lymphocytes: prolonged thoracic duct lymph drainage⁴ during tumour growth; and thymectomy of rats followed by repeated whole-body irradiation⁵, before tumour implantation. Both procedures caused a significant suppression in T-cell dependent immune reactivity, which was demonstrated by the fact that an allograft (Wistar rat)

tumour grew progressively in the treated rats, but was invariably rejected by normal Hooded rats.

Rats immunosuppressed by removal of T cells were inoculated with cells from a syngeneic sarcoma, HSBPA, which when grown in normal animals gives rise to tumours containing approximately 50% macrophages. Figure 1 shows that in the treated rats the mean macrophage content of the tumours was lowered considerably. The rather wide range of variation in the drained rats (Fig 1b) may be a reflection of the total number of lymphocytes removed, since the rate of drainage achieved varied in different animals. The numbers of macrophages in tumours grown in the sham cannulated group were normal, indicating that the stress of surgical trauma and confinement in restraining cages were not responsible for the observed differences. The effect of thymectomy plus irradiation was similar, but the individual values were less widely spread. This may be due to the fact that T cell removal was complete before tumour implantation, whereas in the previous group some degree of host reaction to tumour cells may have been induced before T cells were maximally depleted.

Table 1 Macrophage content, incidence of metastases and immunogenicity of chemically induced rat fibrosarcomata transplanted into syngeneic recipients

Tumour	Mean % macrophages (and range)	Incidence of metastases (%)	Immunogenicity
MC-3	8 (2-12)	100	< 10 ³
HSH	12 (10-15)	100	10 ³ -2 × 10 ⁴
ASBPI	22 (18-26)	50-55	10 ⁵
MCI-M	38 (36-42)	20-30	10 ⁶
HSN	40 (34-44)	30-35	5 × 10 ⁶ -10 ⁷
HSBPA	54 (42-63)	10-12	10 ⁷ -5 × 10 ⁷

The incidence of metastases was measured after excision of tumours which had been growing i.m. in hind limbs for 14 d. Immunogenicity was assessed as the number of cells required for tumour growth in rats which were immunised by excision of i.m. tumours 14 d previously.

tumours to between 9% and 20%. These experiments suggest that the infiltration of macrophages is associated with an effective immune response of the host to the tumour, and that the variation in the macrophage content of different tumours may be related to their immunogenicity.

Immunosuppression has been shown to facilitate metastatic spread^{7,8} and Fig. 2 shows that when the HSBPA rat fibrosarcoma was inoculated into T-cell depleted rats, the incidence of spontaneous metastases was greatly increased. From these experiments it is not possible to deduce whether the simultaneous decrease in macrophage infiltration (see Fig. 1) was causally related to the subsequent increase in metastases. A role for tumour macrophages in the control of tumour dissemination is suggested, however, by the parallel between macrophage content and the rate of spontaneous metastasis of a series of six different rat sarcomas grown in normal syngeneic recipients (Table 1).

The tendency to metastasise is also related (inversely) to the 'immunogenicity' of the tumours—this being defined as the degree of resistance to tumour challenge (expressed as the number of cells required to produce a tumour) in a suitably pre-immunised syngeneic recipient. Immunogenicity is not synonymous with antigenicity, since the former measures the effectiveness of the host response, which is determined both by the magnitude of the immune mechanism and the success of the escape mechanism. Thus the MC3 sarcoma induces cytotoxic cells⁹ and antibody to approximately the same extent as highly immunogenic tumours. The lack of immunogenicity of tumours such as MC3 has been ascribed to a high rate of shedding of tumour-specific antigen into the circulation¹⁰, which combines with and neutralises cytotoxic lymphocytes before they can reach the tumour. The initiation of an immune reaction by itself is, therefore, not sufficient to induce a macrophage infiltration, there must be an effective immune reaction at the tumour site for this to occur. Thus, as in a delayed hypersensitivity site, while the presence of macrophages is an essential component of such a reaction, these cells do not appear until an interaction of immune lymphoid cells with the eliciting antigen has occurred. We have previously shown¹¹ that tumour-bearing animals become progressively less responsive to DHS skin testing antigens and inflammatory stimuli, and it seems that this may be due to a competition of growing tumours for available monocytes, since the magnitude of the defect was directly related to the number of macrophages sequestered in the tumour of the rat being tested.

What then is the role of macrophages in tumours? Are they ineffectual bystanders brought in by lymphokines released in an immune process effected entirely by lymphoid cells interacting with the tumour? Though this remains a possibility, the clear demonstration of an immunologically specific cytotoxicity of macrophages to syngeneic sarcoma cells¹² makes this unlikely and the available data are more

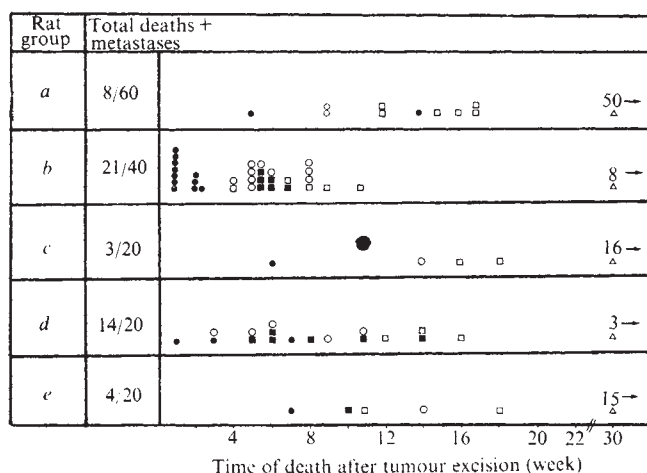


Fig. 2 Effect of immunosuppression on incidence of metastases after excision of HSBPA tumour. HSBPA tumours were grown i.m. in hind limbs of various groups of immunosuppressed rats (lymph drained, *b*, and thymectomised and irradiated, *d*) and appropriate controls (*a*, *c*, *e*) untreated rats *a*, and sham cannulated *c*, or sham thymectomised *c*, for 14 d. The tumour-bearing limbs were then amputated, and the animals kept for observation on the development of metastases for up to 30 weeks. ●, Deaths with no evidence of metastases; ○, deaths with lymph node metastases; □, deaths with lung metastases; ■, deaths with lymph node and lung metastases; △, survivors.

To investigate whether the macrophage content of a tumour can be influenced by raising the immune reactivity of the host, a Hooded rat sarcoma, MC3, was transplanted into August rats. This strain differs from the Hooded strain at minor, but not major histocompatibility loci, and will therefore allow progressive growth of Hooded rat tumours, in spite of a more pronounced host reaction to the grafts. In the syngeneic Hooded rats, the MC3 tumour grows from as few as 100 cells, invariably metastasises, and no protection to a subsequent challenge can be induced by repeated immunisation. In the 'allogeneic combination, (that is, in August rats) larger inocula of 10⁶-10⁸ tumour cells are needed and the tumour remains strictly localised. The average macrophage content of MC3 tumours grown in syngeneic hosts was 8%, whereas in August rats it was raised to 38%.

Non-specific stimulation of the reticuloendothelial system of syngeneic Hooded rats with BCG (Glaxo) before inoculation of MC3 sarcoma cells mixed with PPD reduced the incidence of spontaneous metastases from 100% to 80%, and led to a small rise in the macrophage contents of the

consistent with the view that macrophages in the tumour play an active part in preventing metastatic spread. They may also contribute by phagocytosing tumour breakdown products, thereby reducing the amount of soluble antigen released into the circulation.

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Antibody cell daughters can produce antibody of different specificities

ALTHOUGH it contradicts current dogma, there is considerable indirect evidence for the idea that most antibody diversity is generated only after B cells are stimulated to proliferate by antigens or mitogens¹⁻³. A prediction of this theory, confirmed here, is that there may be rapid production within single clones, of cells producing antibody of different specificities.

We characterised the specificity of antibody from single cells by its cross reactivity, using a modified version of the haemolytic plaque technique⁴. We tested antibody plaque-forming cells (PFC) not against a single type of red cell, but on a mixture of erythrocytes from two different individual sheep. Plaques were either clear (both red cells lysed), partial (only one lysed), or "sombrosos"⁵ with different degrees of lysis of both indicators (Fig. 1). The use of plaque morphology as a specificity marker has been fully justified elsewhere (A. J. C. and L. M. Pilarski, in preparation). It is not influenced by differences in amount of antibody since the morphology of a given plaque is faithfully maintained as it grows (A. J. C. and L. M. Pilarski, in preparation). Nor does it reflect changes in antibody class since, in our experiments, plaques were always direct, produced early in a primary response (1-3 d) and obtained from cultures stimulated with bacterial lipopolysaccharide⁶, all conditions which favour IgM.

Table 1 records that of 911 PFC cultured *in vitro* for 2 d, 93 gave rise to two or more plaque-forming progeny. Procedure B unequivocally established that all PFC came from the initial donor cell. With procedure A, controls showed that the probability of plaque formation by contaminating cells, or by irradiated cells, was very low.

In most cases, plaques produced by all progeny were strikingly homogeneous except for small variations in overall diameter. This seems to serve as a good control of the validity of the marker system. That is, different individual cells from the same clone usually made plaques which were morphologically identical. In 10 cases, however, there was obvious variation in the specificity of the antibody released by daughter cells. Seven of these microclones, (including the six where there were only

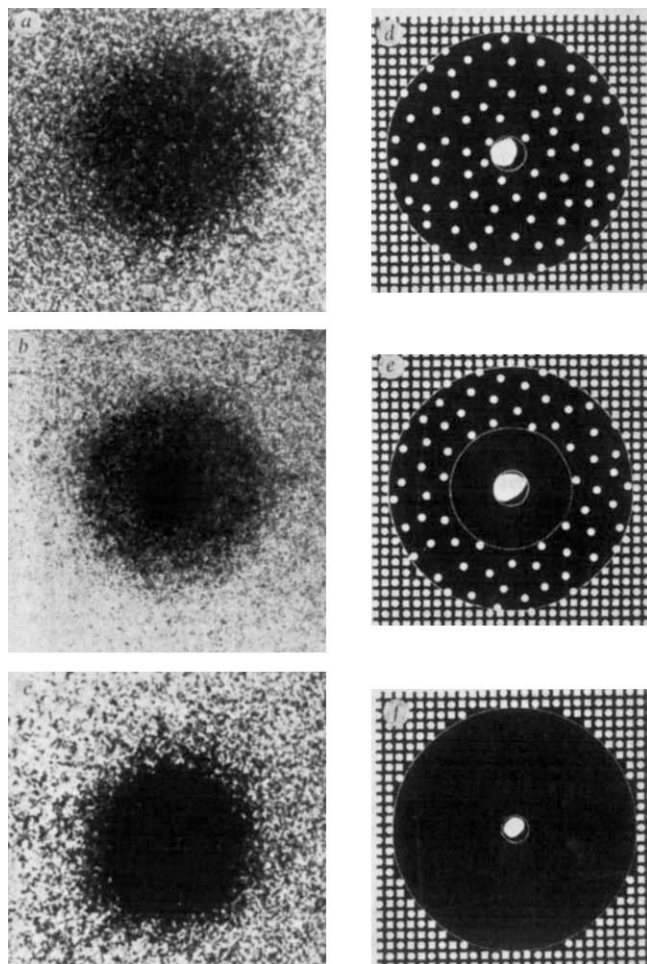


Fig. 1 Photographs (a-c), and diagrammatic representation (d-f), of clear (a, d), sombrero (b, e) and partial (c, f) plaques each produced by a single cell in a mixture of erythrocytes from sheep 1 and sheep 2. Illumination was dark field. In the diagrams, the circles and squares represent red cells of the two different types. A clear plaque is produced by antibody which is equally capable of binding to both indicator red cells. A partial plaque is produced by a different antibody specificity which binds to determinants accessible on one red cell type and not on the other. A sombrero is caused by antibody which binds to both kinds of erythrocyte, but to one better than the other. Mixtures of red cells from two different sheep have been used rather than from sheep and goat or cow as in earlier work^{12,13} so that clear and partial plaques would occur with about equal frequency, to allow the best chance of detecting a change from clear to partial or *vice versa*: the principle is the same whether red cells from two outbred individuals of the same or different species are used.

two plaques) showed two specificity types. Two of the clones contained what seemed to be plaques of three specificities (a clear plaque, a sombrero and partial). The most dramatic example of variation came from the culture of two large and tightly adherent cells (probably daughter cells) taken from the centre of a clear plaque in a microdrop, which on culture yielded a ball of 12 cells. Four of these gave similar plaques with a large clear centre, five produced sombreros with a tiny clear centre, one made a plaque which was possibly intermediate between these two types, one gave an entirely partial plaque, and one was undiagnosed since it was firmly stuck to one of the other cells (see below and Table 2). All of these plaques were similar in overall diameter, and all came from cells which looked macroscopically undamaged and were of similar size.

Sombrero plaques were a common result of these single-cell cultures. The ratio of overall (partial lysis) area to area of clear lysis can vary widely in such plaques, but in most single clones it was strikingly constant: if one plaque had a narrow 'brim' of partial lysis, then all showed exactly the same morphology.

Table 1 Numbers of PFC obtained from cultures of a single initial PFC*

Procedure	Number of individual transfers	Variation shown by clones	Number of cases where x PFC progeny were found												
			x=0	1	2	3	4	5	6	7	8	9	10	11	>11
A	340	No	248	51	18	5	5	1	1	0	1	0	0	0	5†
		Yes			2	2	1								
B	571	No	437	82	30	9	2	2	3	0	0	0	1	0	
		Yes			4										1‡

*Pooled results of all experiments.

†In all of these cases there were one or two large plaques and a large number (up to 60) tiny plaque-like areas with no central white cell, which were possibly caused by fragments of cytoplasm shed from antibody-containing cells.

‡This clone came from two tightly joined cells which were cultured together.

For procedure B, 10^7 spleen cells from one or more normal adult CBA mice were incubated in polyacrylamide rafts, as described by Marbrook and Haskill^{14,15}. Approximately 5×10^6 sterile sheep red cells were added, from any one of several commercial donor sheep. The medium was Eagles minimal essential, plus bicarbonate, with 10% added foetal calf serum. All cultures always contained *Escherichia coli* 0128:B12 lipopolysaccharide (Difco) at a final concentration of $7.5 \mu\text{g ml}^{-1}$. Rafts were incubated at 37°C in an atmosphere of 10% CO_2 and 83% N_2 , at pH 7.3. These cultures were collected at 24 h, when they had developed a total of 100–200 anti-sheep PFC. Cells from these 1 d mass cultures were dispersed under oil in microdrops containing a mixture of red cells from two standard sheep, (1 and 2) together with complement and medium¹⁶. The oil chambers were incubated horizontally for 15–30 min at 37°C , so that plaques could be seen around the rare antibody-forming cells. This period of incubation was kept as short as possible to prevent damage to the cells. For this reason the morphology of the plaque around the donor PFC was not always certain, although there was probably some selection for clear plaques which are easier to see than partials when small. The medium for these short-term incubations in an air atmosphere was 199 plus 10% foetal calf serum, buffered with 0.02 M HEPES. Individual PFC were now isolated from all other cells and micromanipulated out of the drops¹⁶. A single PFC was then transferred with a fine, hand-held micropipette to one of the microculture wells in a new polyacrylamide raft. These wells had each been pre-seeded with about 100 heavily irradiated (5,000 r.) normal CBA spleen cells and 1,000 sterile sheep red cells (again from any one of several commercial donor sheep), the medium being a mixture of equal parts of fresh Eagle's medium and filtered conditioned medium from the donor cultures. The individual PFC was observed at a magnification of $\times 40$ as it was pipetted on to the small pellet in the well. Rafts containing transferred PFC were then incubated for 42–48 h (in rare cases 18–24 h) at 37°C . After culturing, it was possible to identify those cells which had come from the original PFC by their large size, and because they were usually stuck firmly together in small clumps of up to 12 cells. They were sucked out of the well with a micropipette and separated by continuous sucking up and down, where necessary with the addition of trypsin at a final concentration of about $100 \mu\text{g ml}^{-1}$ to the drop for 1–20 min. Not infrequently, two or more cells could not be separated. The cells were then added to a mixture of fresh medium, complement, and red cells from the two standard merino sheep, 1 and 2. The mixture was pipetted into a slide chamber⁴, incubated for 1 h, and the resulting plaques characterised as having clear, partial or sombrero morphology. Procedure A was used for earlier experiments in which a larger number of feeder cells was used and 20–100 random normal cells were transferred and cultured together with the single PFC.

Contrasted with this were the instances of heterogeneous clones. Table 2 records the sizes and relative areas of clear and partial lysis in one representative homogeneous clone, and in two of the clones obtained by procedure B which were classified as showing variation.

The frequency of variation shown by cells in our cultures is obviously much higher than has been previously thought to occur. Assuming an average of two divisions per microclone we estimate about one variation event (mutation or gene switch) per 30 divisions. We have also obtained evidence for a similar rate of variation in limit dilution cultures of spleen cells both *in vitro* and *in vivo* (L. M. Pilarski and A.J.C., in preparation). There are a number of possible reasons why the method we have used has detected many more variants than other methods, the most important being its sensitivity. The plaque technique can pick up a single cell which is different from the rest of a clone. It is also extremely sensitive in that when two closely similar antigens (red cells from two sheep) are used, it seems reasonable that a small change in antibody structure may produce a slight increase or decrease in avidity for one antigen without affecting avidity for the other, resulting in a measurable change in plaque morphology, for example the plaque may change from clear to sombrero. Our conditions (IgM antibody, mitogen stimulation, early stages of clonal development), may also favour variation by comparison with most other clonal work, which has usually been done on protracted cultures of antibody-forming cells or myelomas, (for example refs 7,8). Frequently, the methods used to characterise such antibody as 'monoclonal' would not detect 5% of variants.

The only other attempt to detect clonal variation using the same marker as ours is the work of Nossal and Lewis¹³ who found no variants in the two daughter cells from each of 13 PFC, by testing plaques on a mixture of sheep and goat red cells. According to the rate of variation which we have observed this was too small a number of divisions for a variation event to be expected.

Other groups^{9,10} have recently looked for variation in cultured myeloma cells, using techniques which would probably

Table 2 The morphological characteristics of plaques in three clones, each assayed on the same mixture of red cells from sheep 1 and 2

Clone number	Classification	Diameter* (mm)	Ratio† of areas
1	Homogeneous	0.5	2.4
		0.7	2.0
		0.65	2.1
		0.7	2.0
		0.55	2.2
2	Variation	0.43	2.0
		0.4	7.1
3	Variation	0.55	3.0
		0.50	3.0
		0.50	4.0
		0.55	4.0
		0.50	5.0
		0.60	12.0
		0.38	25.0
		0.45	36.0
		0.55	53.0
		0.45	56.0
		0.53	∞

*Average diameter to which partial lysis extended.

†Ratio of area bounded by border of partial lysis to area of complete lysis.

detect mainly large changes in Ig structure. Nevertheless, Bauml *et al.*⁹ demonstrated a fairly high rate of variation under some culture conditions, leaving open the possibility that small undetected changes in the V region may also have been common.

Our experiments support the idea that antibody diversity can be generated at a high rate after immunocompetent cells are stimulated to proliferate. The experiments describe antibody phenotype only, and do not elucidate the genetic basis for clonal variation. Baltimore¹¹ has recently described a possible mechanism of hypervariation. The small changes in specificity which we observe might well be due to point mutations in the V region (hypermutation), but we cannot entirely exclude rapid switching from one pre-existing gene to another.

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Spontaneous antilymphoma reaction of preleukaemic AKR mice is a non-T-cell killing

THE high incidence of spontaneous leukaemias in AKR mice may be explained by the early appearance and high level of replication of a type C virus. It is often suggested, however, that in addition, AKR mice could be tolerant to their leukaemias. This tolerance would enhance the *in vivo* growth of malignant cells. In fact, AKR mice are not completely devoid of antileukaemic immune response¹⁻⁵. It would be interesting, therefore, to determine if a quantitative or qualitative defect of one given type of antileukaemic response exists in AKR mice. Previous work has shown that an antibody response directed against the type C virus or against antigens of leukaemic cells can be demonstrated not only in hyperimmune¹ but also in normal AKR mice⁴.

A cell mediated reaction directed against G (Gross)⁶ leukaemia antigen-bearing cells was also detected *in vitro*^{3,7} but this cell-mediated reaction was characterised by inhibition of the growth of G+ cells in monolayers. It is now known, however, that this kind of inhibition can be due to non-T cells⁷⁻⁹ and could be antibody dependent¹⁰. A completely different cell-mediated antitumour reaction can be evidenced in the murine sarcoma virus (MSV) induced tumour system by the chromium release test (CRT) which detects a pure T-cell-mediated^{11,12} and antibody-independent¹³ cytolysis of tumour cells. Our work was performed to determine if AKR mice are capable of spontaneously developing such a kind of T-killer cell response.

AKR, C₃H/He, BALB/c and C57BL/6 mice were from our own colony. They have been tested at various ages from 2 weeks to 19 months without any preliminary treatment. Both males and females were used and tested individually. The CRT was done as previously described¹³. The effector cells were spleen cells, and the tumour target cells were lymphoma cells in suspension. A 100:1 effector:target cell ratio was used. The serologically defined tumour antigens and the origin of the target cells are given in Table 1. The specific chromium release was measured after 18 h incubation of target cells in the presence of effector cells. It was expressed as the excess of release when target cells were incubated with AKR spleen cells in place of C₃H/He, BALB/c or C57BL/6 control cells. No difference in the chromium release can be detected when non-AKR cells were used as effector, whatever the age of the donor mice. As previously discussed, an excess of 4% in the chromium release can be considered as significant¹³.

Results of experiments in which the effectors were whole spleen cells are given in Table 1. Individual AKR mice less than 3 months old were constantly negative. Positive CRT was found in 75% of 3-5-month-old AKR mice. The reaction was generally weak (specific release average = 12%), but could occasionally reach a high level (20-30%). Not only syngeneic or allogeneic G+FMRGi- lymphoma cells, but also G-FMRGi+ lymphoma cells could be lysed by the AKR spleen cells. Such a cross reaction was previously observed in MSV tumour system^{13,14}. ERLD lymphoma and P815 mastocytoma, which bear no serologically defined leukaemic antigen⁵, were never lysed by AKR spleen cells. Leukaemia of AKR mice began to appear after 5 months. Positive CRT reactions were observed in 41% of non-leukaemic animals older than 5 months. These results show that positive reactions are less frequent than in younger mice. In addition, positively-reacting animals were more frequent between 5 and 8 months (57%) than after 8 months (30%). CRT reactions were always negative in mice with overt leukaemia (enlargement of spleen, lymph nodes and thymus).

Table 1 Frequency of spontaneous reactions of AKR in CRT

Effector spleen cells	(G+) target cells*			Total of experiment with (G+) target	(G-) target cells*		
	GL 3 (G+FMRGi-)	E δ G2 (G+FMRGi-)	SK 1 (G+FMRGi-)		GiL 4 (G-FMRGi+)	ERLD (G-FMRGi-)	P 815 (G-FMRGi-)
2 weeks to 3 months old	0/32†	0/6	0/12	0/40	0/6	0/7	0/5
3-5 months old	26/35	3/4	4/5	33/44	6/10	0/3	0/7
Non-leukaemic: older than 5 months	21/46	1/4	5/13	27/63	3/12	0/23	0/3
Leukaemic: older than 5 months	0/14	0/2	ND†--	0/16	0/4	0/7	ND

*GL 3 and E δ G2 = C57BL/6 Gross virus-induced lymphoma.

SK 1 = AKR spontaneous lymphoma.

GiL 4 = C57BL/6 Graffi virus induced lymphoma.

ERLD = C57BL/6 X-ray induced lymphoma.

P 815 = DBA/2 methyl-cholanthrene induced mastocytoma.

†Number of mice with positive results/number of tested mice.

‡ND = Not done.

Table 2 Effect of anti- θ AKR serum and complement treatment on the activity of spontaneously cytotoxic AKR spleen cells for the GL 3 lymphoma of C57BL/6 mice

Spleen cells from AKR	Specific chromium release (%) in the presence of reactive spleen cells treated by:				
	Medium alone	C ₃ H normal serum with complement	C ₃ H normal serum without complement	C ₃ H anti- θ AKR serum with complement	C ₃ H anti- θ AKR serum without complement
4 months old	25	23	22	27	28
5 months old	19	17	20	22	21
6 months old	11	12	9	13	12
7 months old	8	7	8	10	7
8 months old	15	12	13	14	13

These results suggest that AKR mice can develop a spontaneous antileukaemic reaction which appears during the preleukaemic period and completely disappears with the progression of the disease. It was previously shown that the CRT allows the detection of a pure T-killer cell phenomenon in our MSV tumour system^{11,12} as well as during allograft rejection^{15,16}. Thus, we have attempted to discover whether T cells could be responsible for the spontaneous CRT reactions detected in AKR mice. The whole spleen cell suspensions of individual AKR mice were treated with C₃H/He anti- θ AKR serum diluted at 1:10 and rabbit complement, before they were used in the CRT. Anti- θ AKR serum was obtained by repeated inoculations of AKR thymocytes in C₃H/He mice, the serum was absorbed by C₃H/He thymocytes and tested for its specificity for various lymphoid organs of AKR mice. Controls included in each experiments were first, AKR spleen cells treated by normal C₃H/He serum and rabbit com-

graft. It can be concluded that the positive results obtained in the CRT with AKR spleen cells were due to non-T cells.

To study the nature of the effector cells, spleen cell suspensions from individual AKR mice were treated according to one of the following procedures (Table 4): first, incubation for 30 min at 37° C with carbonyl iron followed by six successive passages over a powerful magnet¹⁸. This procedure removes practically all the macrophages. Second, filtration through anti-immunoglobulin-coated glass bead columns according to the method of Wigzell *et al.*¹⁷. This method removes both the macrophages and the immunoglobulin-bearing lymphocytes, and the eluted suspensions contain about 90% T cells. Third, incubation for 20 min at 37° C with 0.25% trypsin. The reaction was stopped by the addition of five volumes medium containing 10% foetal calf serum. This method allows the eventual removal of antigen-antibody complexes attached to effector cells. The results (Table 4) show that column filtration

Table 3 Effect of anti- θ AKR serum and complement treatment on the activity of anti-C57BL/6 immune AKR spleen cells for the GL 3 lymphoma of C57BL/6 mice

Anti C57BL/6 immune spleen cells from AKR	Specific chromium release (%) in the presence of immune spleen cells treated by:				
	Medium alone	C ₃ H normal serum with complement	C ₃ H normal serum without complement	C ₃ H anti- θ AKR serum with complement	C ₃ H anti- θ AKR serum without complement
2 months old	31	38	35	0	33
5 months old	23	26	24	0	27
8 months old	36	37	34	0	38

plement and, second, AKR spleen cells treated by anti- θ AKR serum without complement.

The results of a typical experiment are summarised in Table 2. They show that the elimination of θ antigen-bearing cells does not decrease the antileukaemic response of AKR spleen cells. Twenty-three positive mice were individually studied giving identical results. It is unlikely that these results would be a consequence of a weak activity of the anti- θ AKR serum used, since this serum lyses all T-cells of AKR mice at dilutions up to 1:100 and because AKR receiving 4×10^7 C57BL/6 spleen cells intraperitoneally have cytotoxic lymphocytes in CRT for C57BL/6 target cells and pretreatment of these cytolytic cells with anti- θ AKR serum diluted at 1:10 and rabbit complement abolish their activity (Table 3). In addition, this last result demonstrates clearly that AKR mice are perfectly able to develop a T-killer cell response against an allogeneic

strongly decreases the CRT activity of spleen cells, and carbonyl iron treatment abolishes it completely. Identical results were obtained with the 18 reactive mice tested. It can be concluded that non-T cells, probably macrophages, are the main effector cells of the spontaneous CRT reactions of preleukaemic AKR mice. Treatment of spleen cells with trypsin suppress the CRT activity (Table 4), and this activity does not reappear even 24 h after trypsinisation. It is likely, therefore, that an antibody-dependent cell-mediated reaction is involved. We are studying the capacity of various AKR sera to block reactive cells and to 'arm' nonreactive cells in CRT.

Three points should be noted. First, an antileukaemic reaction can be detected by the CRT in preleukaemic AKR mice, but this reaction, probably antibody mediated, is unable to protect the mice since it was found in at least 75% of 3-5-month-old AKR mice, whereas the incidence

Table 4 Effect of various treatments on the cytotoxic activity of reactive AKR spleen cells

Spleen cells from AKR	Specific chromium release (%) in the presence of reactive spleen cells treated by:			
	Medium alone	Column filtration	Carbonyl iron and magnet	Trypsin
4 months old	11	0	0	0
4 months old	21	7	0	0
5 months old	16	4	0	ND
7 months old	12	0	0	0
7 months old	9	0	ND	0
8 months old	8	0	0	0

of leukaemia in AKR mice is about 80%.

Second, we have demonstrated^{11,12} in the MSV tumour system that the CRT allows the detection of pure T-cell killing of syngeneic tumour cells. Similar observations were done previously in allogeneic immunisation¹³⁻¹⁶. In the same experimental conditions, however, the AKR spontaneous reaction is caused by non-T cells. Immune cytotoxicity of P 815 tumour cells resulting from non-T cells have been also detected in old NZB mice¹⁸. Furthermore, it is known that in the microtoxigenicity assay (MA) both T and non-T cells^{7,8} and even macrophages⁹ can be involved. It therefore seems necessary to determine precisely the nature of the effector cells for each tumour system, whatever the *in vitro* methods used to study the cell-mediated immunity.

Third, the absence of T-killing in the antileukaemic response in preleukaemic AKR mice must be emphasised since the same animals can respond normally to allogeneic grafts with a high level of specific T-killer cells. The existence of such a reaction not only in young AKR but also in 8-month-old mice is especially interesting and suggests that a decrease with aging in the immune competence of AKR is not involved. It is noteworthy that the same AKR mice which develop anti-allogeneic T-killer cells can have at the same time a non-T cell mediated reaction directed against syngeneic G+ lymphoma. The first reaction is abolished by anti- θ AKR whereas the second remains unchanged. Preliminary results indicate that the absence of T-killer response is not a general property of the leukaemia viruses since resistant mice such as C57BL/6 are able to develop a 'T' response after Friend or Moloney virus inoculation (unpublished data). Further experiments in the Gross system using both sensitive and resistant mice are now in progress to determine whether the absence of T-killer cell response is a property of AKR mice or not. A hypothesis would be that the *Rgv-1* gene¹⁹ which conditions the sensibility to Gross virus induced leukaemias could be responsible for the AKR inability to develop an antileukaemic T-killer cell response. This hypothesis could be reinforced by the fact that an H-2-linked gene, possibly identical to *Rgv-1* mapped in the H-2 complex near the 'Ir region'²⁰ has been recently shown to act like an immune response control gene²¹.

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Observations on the mechanism by which T-lymphocytes exert cytotoxic effects

THE mechanism by which T lymphocytes kill tumour cells is unknown¹. The killing is more rapid than reported for lymphotoxin². Among possible mechanisms are the involvement of complement components³ or phospholipase A, which could generate lysolecithin in the target cell membrane. To test these possibilities we have examined the rates of release from tumour cells of markers of high and low molecular weight and the effects of nonpenetrating solutes. If killing involves disruption of the structure of the cell membrane, for example by lysolecithin, simultaneous release of markers and no protection by macromolecular solutes would be expected. If lysis is osmotic, markers of low molecular weight should be released before those of high molecular weight and nonpenetrating solutes should protect against the lysis by counterbalancing the intracellular osmotic pressure. In the case of complement lysis, small macromolecules of molecular weight less than 40,000, which

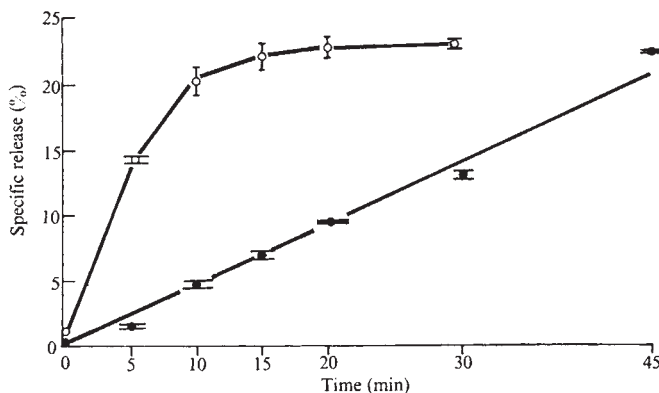


Fig. 1 Time course of the specific release of ⁸⁶Rb (O—O) and ⁵¹Cr (●—●) from mastocytoma cells by sensitised spleen cells. P-815-X2 mastocytoma cells were double labelled: 5 × 10⁶ cells were incubated for 1 h at 37° C with 100 μCi of Na⁵¹CrO₄ and 1000 μCi of ⁸⁶RbCl in 1 ml Eagle's minimal essential medium containing 10% foetal bovine serum. Sensitised spleen cells were obtained from C57BL/6 mice injected intraperitoneally 11 d earlier with 3 × 10⁷ live mastocytoma cells. Mixtures containing 10⁷ normal or sensitised spleen cells and 1.5 × 10⁵ labelled mastocytoma cells in 1 ml of the above medium were centrifuged in flat bottomed plastic tubes of 1.25 cm diameter and incubated at 37° C for the indicated lengths of time. Then they were mixed, briefly centrifuged and aliquots of supernatants taken for counting the released labels. Percentage specific release of the two labels was obtained by subtracting the release in the presence of normal spleen cells from the release in the presence of sensitised cells and is expressed as a fraction of the total labels released by freezing and thawing the cells. The values are means of duplicates and the range of variation is indicated.

penetrate through complement lesions, do not inhibit lysis, whereas those of molecular weight above 40,000 protect⁴.

Our experiments have been performed with a widely studied cytotoxicity model, mastocytoma cells from DBA/2 mice and sensitised lymphocytes from C57BL/6 mice¹. As a convenient small marker we have used ⁸⁶Rb⁺, which is not bound in the cytoplasm and behaves in a manner analogous to K⁺, and as a large marker ⁵¹Cr which is bound to cytoplasmic protein. The time course of release of the two markers is shown in Fig. 1. It is clear that the specific release of ⁸⁶Rb⁺ occurs much more rapidly than that of ⁵¹Cr, as previously reported by Henney³. In contrast to Henney³, but like C. J. Sanderson and G. A. Taylor (unpublished), we find linear release of ⁵¹Cr from the time of contact of effector lymphocytes and target cells. The slope of the curve

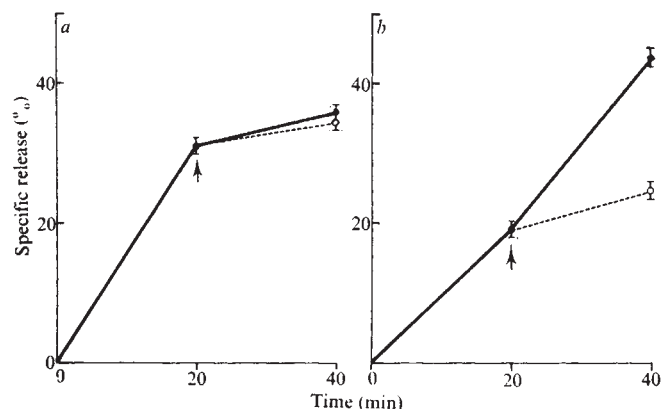


Fig. 2 Effect of dextran on the specific release of a, ⁸⁶Rb and b, ⁵¹Cr from mastocytoma cells by sensitised lymphoid cells. Spleen cell-target cell mixtures ($10^7 : 1.5 \times 10^5$ in 0.5 ml) were centrifuged and incubated at 37° C for 20 min. At that point (shown by arrow) 0.5 ml of a solution of 24% dextran molecular weight 10,000 daltons was added to some 0.5 ml cell suspensions and all samples were incubated for a further 20 min before the release of the two labels was assessed in the presence (○) or absence (●) of dextran.

of ⁵¹Cr release is related in a linear fashion to the concentration of the effector cells. These observations allow three conclusions. First, the cytotoxic mechanism depends on contact of one effector cell with a target cell. Second, within minutes of the establishment of such a contact the plasma membranes of the target cells show increased permeability to ions but not proteins. Third, loss of cytoplasmic proteins follows a slower, linear time course beginning in some cells within a few minutes of contact and occurring in other cells only after a delay of an hour or more.

Although several nonpenetrating solutes were found to inhibit the lysis of target cells, most experiments were performed with dextran of molecular weight 10,000 (T 10). Representative experiments with dextran T 10 in the extracellular medium are shown in Table 1 and Fig. 2. When the dextran was added at the beginning of the experiment, inhibition of the release of ⁸⁶Rb as well as of ⁵¹Cr was found, presumably because effective contact of the lymphocytes and target cells was prevented. When dextran was added after 5 min there was only slight inhibition of ⁸⁶Rb release (possibly because effective contacts between lymphocytes and all target cells had not yet been established) but there was much greater (70%) inhibition of ⁵¹Cr release. Addition of dextran as late as 20 min still produced highly significant inhibition of ⁵¹Cr release but none of ⁸⁶Rb release. This effect was fully reversible: when medium containing dextran was replaced with medium lacking dextran, the release of ⁵¹Cr proceeded rapidly to completion. Hence the presence of dextran does not affect the increased permeability of target cell membranes to ions but produces a marked and reversible inhibition of the release of cytoplasmic proteins.

Table 1 Effect of dextran on release of ⁵¹Cr and ⁸⁶Rb from mastocytoma cells by sensitised lymphoid cells

Experiment No.	Time (min) when dextran added	Isotope released	% inhibition of specific release	
			15-20 min*	40-45 min
1	5	⁵¹ Cr	70 ± 1.7	44 ± 1.1
1	5	⁸⁶ Rb	14 ± 1.8	0 ± 1.4
2	15	⁵¹ Cr	53 ± 1.9	51 ± 1.6
2	15	⁸⁶ Rb	0 ± 2.1	0 ± 2.0
3	20	⁵¹ Cr	37 ± 0.7	30 ± 0.6
3	20	⁸⁶ Rb	0 ± 0.9	0 ± 1.1

*Time after dextran addition.

The cytotoxicity assays were carried out as in Fig. 2, but with varying time intervals before and after the addition of dextran solution to the cell mixtures

The kinetics of release of large and small markers and the protection against loss of large markers by low molecular weight dextran provide strong evidence that the lysis of tumour cells by sensitised T lymphocytes occurs through osmotic effects and that the ion-conducting channels formed in the plasma membrane of the target cells are smaller than those produced by complement. As shown in Table 2, neither the osmotic lysis produced in the same target cells by antibody and complement nor that produced by lysolecithin is appreciably affected by dextran of molecular weight 10,000.

It has been reported that cooling the injured target cells to 0° C in the effector cell independent phase of the reaction prevents ⁵¹Cr release⁶. This observation was interpreted as evidence in support of an enzymic reaction. An alternative explanation is that because of loss of fluidity in the target cell membrane the ion-conducting channels generated following contact with effector lymphocytes are sealed at low temperatures. As shown in Fig. 3, cooling to 10° C inhibits almost completely leakage of ⁸⁶Rb as well as ⁵¹Cr from the target cells. In other experiments ⁸⁶Rb and ⁵¹Cr release were markedly depressed even at 15° C, and returned to control levels when the cells were placed again at 37° C. These observations support the view that membrane fluidity is

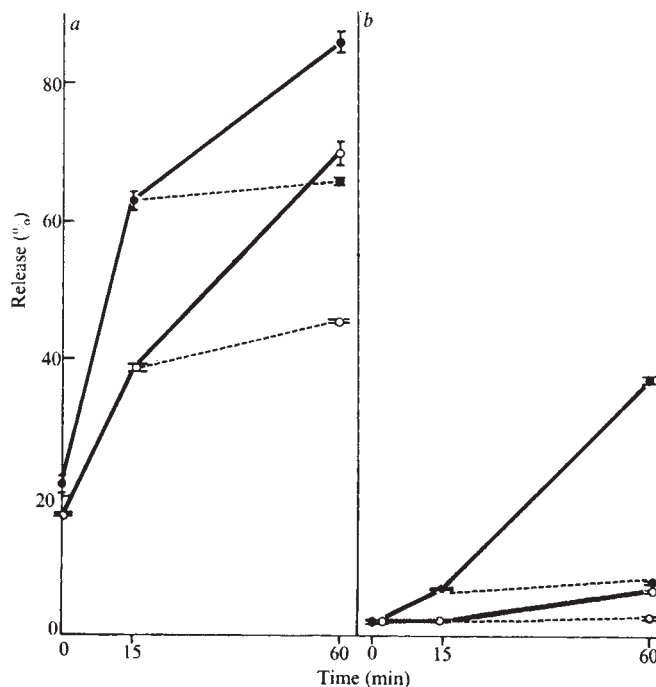


Fig. 3 Effect of lowering temperature on a, ⁸⁶Rb and b, ⁵¹Cr release from the mastocytoma cells. Spleen cell-target cell mixtures were prepared as in Fig. 1 and incubated at 37° C for 15 min. Then some cell samples were cooled to 10° C and all cell samples were incubated at the appropriate temperatures for up to 60 min. The labels released from the target cells in presence of sensitised (●) or normal (○) spleen cells at 37° C (—) or at 10° C (---).

involved. Even the basal release of ^{86}Rb in the absence of specific effector cells is markedly reduced at 10° – 15°C , which is again consistent with a requirement for membrane fluidity.

These observations help to clarify the mechanism by which sensitised T lymphocytes lyse target cells. The first stage is the establishment of effective contact between effector cells and target cells. This requires effector cells which are living and moving: inhibition of motility of the cells by cytochalasin^{7,8} blocks the early but not the later stages of the reaction. The inhibition of target cell lysis by agents generating cyclic AMP^{9–11} may exert their effects by depressing movements of effector cells. Movement is required for three reasons: bringing effector cells and target cells together, moving apposed membranes to establish intimate contact of appropriate type, and subsequent movement of the cells apart. Once effective contact of lymphocytes and target cells is established (which begins within a few minutes of their coming together) killing the lymphocytes by specific antibody and complement does not prevent continued lysis of target cells: intact effector cells are not required for the final lytic process¹². In fact, the films of Ginsburg and Ax¹³ show that lysis does not take place while the effector cell and target cell are in contact but occurs only after the effector cell has disengaged itself and moved on. The films also show that the diameter of mastocytoma cells is increased before lysis, which is consistent with the interpretation that osmotic swelling is taking place.

Table 2 Effect of dextran on the lysis of tumour cells by antibody and complement and by lysolecithin

Lytic agent	Dextran	% Specific ^{51}Cr release	% Inhibition by dextran
Antibody	---	91 $\pm$ 0.9	—
Antibody	T 10	86 $\pm$ 0.2	6
Antibody	T 40	46 $\pm$ 2.3	50
Lysolecithin	---	100 $\pm$ 0.2	—
Lysolecithin	T 10	100 $\pm$ 0.1	0

Tumour cells were incubated with a minimum lytic concentration of rabbit antiserum against mastocytoma cells and guinea pig complement (4 haemolytic units) or with lysolecithin ($20\text{ }\mu\text{g ml}^{-1}$) for 1 h at 37°C . Lysis in the presence and absence of dextran (12%) was compared. The ^{51}Cr release by antibody was compared with that produced by freezing and thawing cells, and that by lysolecithin, which was higher, compared in the presence and absence of dextran.

During the second phase of the reaction the plasma membrane of the target cell becomes leaky to ions. The underlying mechanism is not yet certain. An appealing hypothesis is that a gap junction is established between the effector and target cell. The report of Sellin and his colleagues¹⁴ that fluorescein passes from target cells to sensitised lymphocytes supports this interpretation. The electrotonic coupling, involving free passage of ions, of lymphocytes treated with phytohaemagglutinin¹⁵ is also relevant since this lectin can promote lysis following contact between effector cells and target cells¹⁶. Gap junctions are characterised by clusters of particles in apposed membranes demonstrable by freeze-fracturing electron microscopy¹⁷, and it is conceivable that crosslinking of proteins in the membrane of the target cell by specific receptor molecules on the surface of the lymphocyte (by a process analogous to patching¹⁸) may be involved in the generation of gap junctions, and also in the persistence of such leaky complexes, allowing increased passive ion flux in the membrane of the target cell once the lymphocyte has moved away.

The experiments reported in this paper seem to exclude a role of lysolecithin generation or of typical complement-induced membrane lesions in the lysis of tumour cells by sensitised T lymphocytes. A second phase of lysis, which does not require the presence of intact effector cells, is characterised by increased permeability of the target cell

membrane to ions but not to cytoplasmic proteins, and the final lytic event is due to osmotic effects. The nature of the lesion in the target cell membrane is not yet certain, but it may be due to the formation and persistence of a gap junctional complex.

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Rapid mixed lymphocyte culture test based on relative increase in protein synthesis

We have developed a mixed lymphocyte culture assay for matching prospective recipients of organ transplants to donor cadavers. Its rapidity, combined with the use of frozen cells from a recipient panel, could facilitate the sharing of cadaveric organs among transplantation centres.

Allogeneic lymphocytes mixed in culture stimulate each other and undergo blastogenesis to a degree proportional to the degree of antigenetic disparity between the cells. This mixed lymphocyte reaction (MLR) is a precise means by which the graft *versus* host response can be estimated *in vitro*. MLR is usually evaluated by the measurement of DNA synthesis in 5–8-d-old mixed lymphocyte cultures (MLC). Although the MLC test has been used for the study of some aspects of histocompatibility in man, its use for matching donors to recipients for cadaveric organ transplantation has been limited by the length of the test. A more rapid test (72 h) measuring stimulation of DNA synthesis in MLC has been reported¹, and a method for early evaluation (11–24 h) of mixed leukocyte interaction in mice and

Table 1 MLR after inhibition of protein synthesis in stimulating lymphocytes

Lymphocytes	c.p.m. ^3H -leucine 1 h pulse	c.p.m. ^3H -thymidine 8 h pulse (5 d culture)
C	1220	307
D	1170	342
D _m	1008	96
D _e	112	42
D × D _e	1216	324
C × D	6978	2176
C × D _e	1357	298
C × D _m	5109	1585

The effects of emetine on stimulation of DNA and protein synthesis in MLR. Suspensions of lymphocytes (C) and (D) were prepared as described in the text (2×10^6 cells ml^{-1}). Lymphocytes D were arbitrarily selected as stimulating cells and were treated with $25 \mu\text{g ml}^{-1}$ mitomycin C for 30 min at 37°C and used in MLC after excess mitomycin was removed by three washes with medium. Similarly, lymphocytes D were treated with 1 mg ml^{-1} emetine for 30 min and used as D_e after three washes. Both mitomycin-treated (D_m) and emetine treated (D_e) cells remained viable (97%) as tested by trypan blue exclusion. Lymphocytes C and D (D_e and D_m) were incubated alone or mixed together and incubated in leucine-deficient medium in the presence of ^3H -leucine $0.001 \mu\text{mol ml}^{-1}$ for 1 h, collected and counted for ^3H incorporation. They were also cultured alone or mixed together and cultured for 5 d in chemically defined medium³ containing 5% calf serum. On the fifth day cells were collected 8 h after the addition of $10 \mu\text{Ci ml}^{-1}$ of ^3H -thymidine. Incorporation of ^3H -thymidine was measured by scintillation counting. A mixture of DD_e was used to rule out possible carry-over of excess emetine by D_e.

guinea pigs based on the measurement of protein synthesis has been described²⁻⁴. We now report a rapid MLC assay (1 h) to discriminate between isogenic and allogeneic human lymphocytes by measuring the relative increase in the rate of protein synthesis in MLR. The assay allows the use of frozen lymphocytes from a panel of prospective recipients.

Using a synthetic medium⁵ free of leucine, we compared the changes in the rate of ^3H -leucine incorporation into proteins of allogeneic lymphocytes. Leukocyte-rich plasma was obtained from heparinised blood after red blood cell sedimentation, and the leukocytes were isolated by centrifugation (10 min at $150g$). Lymphocytes were separated from granulocytes and red blood cell contaminants by discontinuous density gradient centrifugation⁶.

Protein synthesis was measured as described in the legend to Fig. 1. The first increase in protein synthesis of MLR appeared as a distinct increase in the rate of ^3H -leucine incorporation beginning as early as 15 min after lymphocytes were mixed in culture. This increase lasted for about 4 h and then declined to a rate more characteristic of unstimulated lymphocytes. The second phase, probably more intimately related to DNA synthesis, appeared about 12 h after the initial contact of allogeneic lymphocytes in culture. This phase seems to correspond to the stimulation of protein synthesis reported in MLR of guinea pigs and mice²⁻⁴.

The first phase of stimulation constituted the basis for the test. Lymphocytes were separated from their medium by filtration and cellular proteins and the proteins released

into the medium by the lymphocytes were isolated. The patterns of leucine incorporation into these proteins after electrophoresis on polyacrylamide gel⁷⁻⁹ were compared with those of isogenic lymphocytes in culture (Fig. 2). The results indicate that a major portion of the increase of radioactivity in MLC is associated with the proteins excreted into the medium. The pattern of ^3H -leucine incorporation into proteins of allogeneic lymphocytes changes within 1 h of initial contact between these cells. The appearance of new and high peaks of leucine uptake suggests the synthesis of new proteins in stimulated cells.

To confirm the participation of both cell types in the

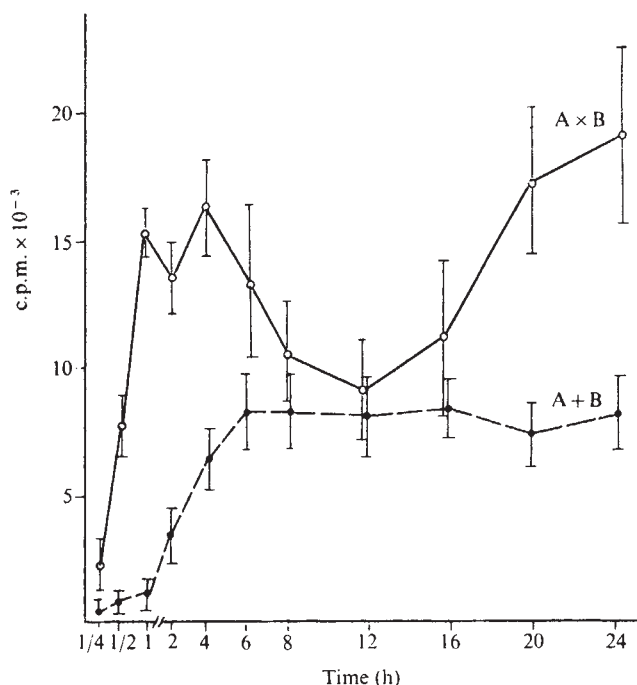


Fig. 1 Biphasic protein synthesis in MLR: protein synthesis was measured by uptake of ^3H -leucine into acid-precipitable proteins. Allogeneic lymphocytes A and B (2×10^6 cells per ml) were incubated in a medium with low concentrations of leucine ($1 \mu\text{mol l}^{-1}$) by themselves or mixed (A:B=1) at 37°C for up to 24 h. Ten cultures of each type (A, B and A × B) were collected at each time point. For the first three time points (15, 30 and 60 min), $0.001 \mu\text{mol ml}^{-1}$ of ^3H -leucine was added to each culture at the plating time and cultures were collected after 15, 30 and 60 min of incubation. Other cultures were collected at 2–4 h intervals 1 h after the addition of $0.001 \mu\text{mol ml}^{-1}$ of ^3H -leucine. The total acid-precipitable protein of the medium and cells of each culture was collected on Millipore filters ($0.2 \mu\text{m}$ pore size), dried and counted in a scintillation counter. ○, Mean c.p.m. of allogeneic MLC (A × B); ●, 1/2 mean c.p.m. of lymphocyte A + 1/2 mean c.p.m. of lymphocyte B (A + B). The bars indicate ± 1 s.d.

first phase of protein synthesis, one cell type was pretreated with emetine¹⁰⁻¹², which irreversibly inhibits cell protein synthesis without affecting viability. This prevented stimulation of protein and DNA synthesis in allogeneic lymphocytes in MLC (Table 1), strongly suggesting that active protein synthesis in both the stimulating and responding cells is necessary for MLR. This reaction, at least in the early phase of MLC, is a two way response.

In our leucine-free medium, the addition of a minimum of $0.001 \mu\text{mol ml}^{-1}$ of labelled leucine was required for an adequate response (Fig. 3). Different degrees of uptake of ^3H -leucine occurred when the leucine concentration was varied. Detection of stimulation of protein synthesis in a given pair of lymphocytes was optimal with $0.001 \mu\text{mol l}^{-1}$ of leucine (Fig. 3).

We have compared the stimulation ratio (c.p.m. of MLC per c.p.m. of individual allogeneic lymphocytes in culture)

Table 2 Correlation of stimulation ratio in rapid and standard MLC

Lymphocytes in MLC	Stimulation ratio	
	DNA synthesis	Protein synthesis
A × Z	3.7	2.7
B × Z	4.0	3.2
C × Z	6.2	4.8
D × Z	9.6	10.0
E × Z	18.5	15.6

Correlation of protein synthesis in rapid MLC with DNA synthesis in 5 d MLC. Triplicate cultures were prepared described in Table 1, and stimulation ratios were calculated as follows c.p.m. of A × Z/1/2 c.p.m. of A + 1/2 c.p.m. of Z and so on. Lymphocytes A, B, C, D and E showed similar degrees of stimulation of DNA and protein synthesis by cell Z in two-way reactions.

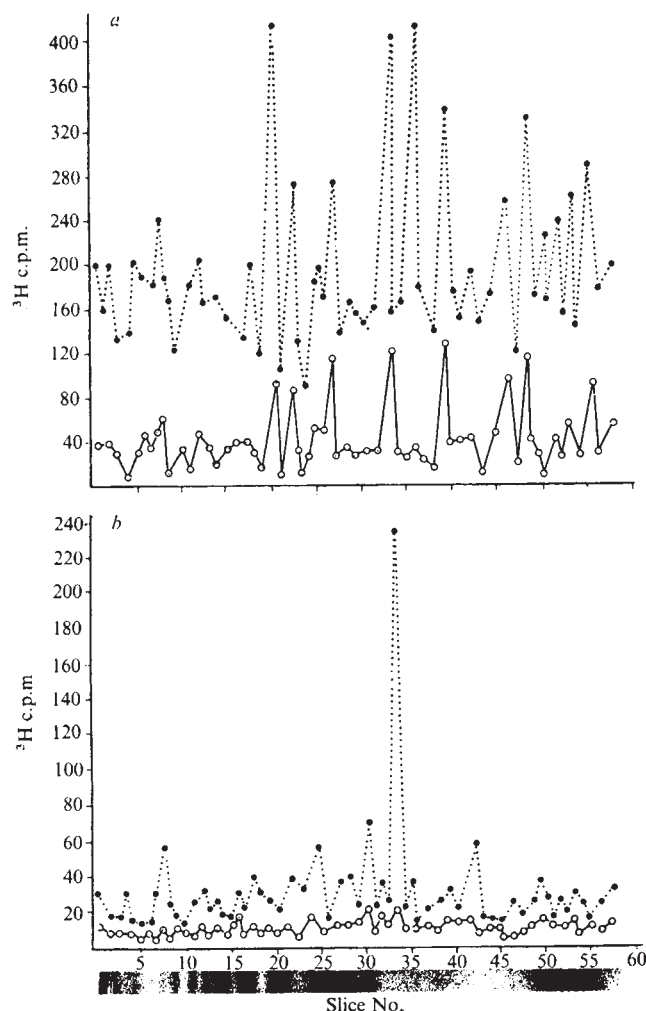


Fig. 2 Electrophoretic patterns of ^3H -leucine-labelled proteins of stimulated (●) and unstimulated (○) lymphocytes. Lymphocyte cultures were prepared as described in Fig. 1: $0.001 \mu\text{mol ml}^{-1}$ of ^3H -leucine was added to each culture at the time of plating and incubated for 1 h. Lymphocytes were separated from their culture medium by filtration through a Millipore filter ($0.45 \mu\text{m}$ pore size). Cells were suspended in 0.4 ml of 0.05 M Tris-HCl, $\text{pH } 7.4$, and mixed with 0.8 ml of 2% sodium dodecyl sulphate (SDS) solution containing 6 M urea and 25% mercaptoethanol and dissociated by heating at 100°C . Similarly, 0.4 ml of medium was mixed with 0.8 ml of 2% SDS containing 6 M urea and 25% mercaptoethanol and dissociated by heating at 100°C . Aliquots (0.1 ml) of each denatured solution were applied to 7.5% acrylamide gel columns prepared according to Russell and Skehel⁷⁻⁹ and electrophoresed for 8 h at 3 mA per gel. Gels were washed in 7% acetic acid and sliced 1 mm thick, each slice was solubilised in 0.1 ml of NCS (Nuclear Chicago) and counted in a scintillation counter after addition of counting fluid. *a*, Electrophoresis of proteins released into medium: ●, c.p.m. of stimulated lymphocytes (A × B); ○, c.p.m. of unstimulated lymphocytes (A + B); *b*, electrophoresis of cellular proteins: ●, c.p.m. of stimulated lymphocytes (A × B); ○, c.p.m. of unstimulated lymphocytes (A + B).

of protein synthesis in rapid MLC with the stimulation ratio of DNA synthesis in 5-d MLC of 500 related and 100 unrelated donor-recipient pairs. The stimulation of DNA synthesis in all cases correlated well with the increase in the rate of protein synthesis in rapid MLC (Table 2).

This method was used to pair prospective kidney transplant recipients and cadaveric donors. Frozen cells from recipients were prepared and stored at -80°C in four panels according to their blood types (A, B, AB or O). Lymphocytes from cadaveric donors of a given blood type

Table 3 Correlation of Rapid MLC with graft rejection

Number of cases	Rejection	Stimulation ratio	
		Range	Mean
4	0	1.2–1.8	1.65
8	1+	2.1–3.8	2.60
6	2+	3.4–4.8	4.05
3	3+	6.7–10.4	8.10
17	4+	3.8–24.8	11.60

Correlation of stimulation ratio in rapid MLC with graft rejection. Thirty-eight recipients of cadaveric kidneys were selected and transplanted on the basis of negative cross match. The stimulation ratios in rapid MLC were measured as described. Rejection was classified as follows: 0, no rejection—normal function (serum creatinin less than $1.5 \text{ mg}\%$); 1+, one rejection crisis, reversed—normal function; 2+, two rejection crises, reversed—less than normal function (serum creatinin more than $1.5 \text{ mg}\%$); 3+, two or more rejection crises, but kidney lost after 3 months; 4+, uncontrollable rejection requiring graft nephrectomy.

(A, B, AB or O) were mixed with lymphocytes of each recipient in the corresponding panel for the rapid MLC test. Thus a gradient of stimulation ratios was obtained, and the donor-recipient pair with the lowest stimulation ratios was selected. Stimulation ratios among a panel of recipients tested against lymphocytes of a cadaveric donor ranged between 2 and 30.

The rapid MLC test allows rapid selection of a suitable donor-recipient pair from a recipient panel based on the MLR. For the selection of donor-recipient pairs on this basis, however, the panel would have to contain a large pool of recipients to allow for the exclusion of those donor-recipient pairs that have a low stimulation ratio of protein synthesis but a positive cross-match. Our preliminary study

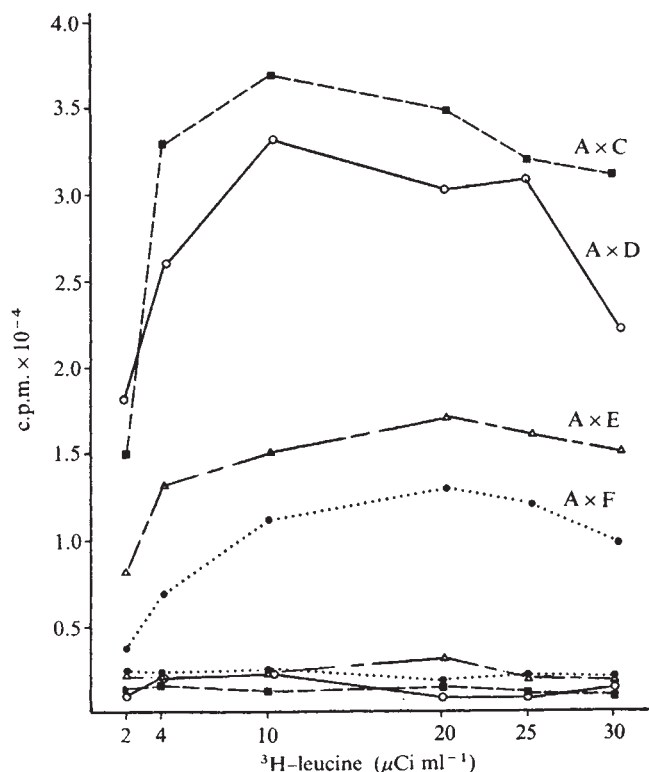


Fig. 3 The effects of variation of leucine concentration on detection of MLR. Allogeneic lymphocytes C, D, E and F were prepared as described in the text and suspended in leucine-free medium (2×10^6 cells per ml) in triplicate. They were incubated by themselves or mixed with lymphocytes A (2×10^6 cells per ml) for 1 h in the presence of variable amounts of ^3H -leucine. Radioactivity of incorporated leucine was measured as described for Fig. 1. Mean c.p.m. of stimulated lymphocytes are shown as A × C, A × D and so on. The lower curves represent $1/2$ (mean c.p.m. A + mean c.p.m. B) and so on.

in 10 cadaveric transplants suggested that the degree of survival of the renal transplant is inversely related to the magnitude of the stimulation ratio in rapid MLC¹². Present data (Table 3) confirms our previous findings.

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Use of immune lymphocytes to detect expression of herpetic genome

HERPES simplex virus DNA and RNA have been demonstrated in one specimen of cervical carcinoma¹ but these experiments are very difficult to reproduce, possibly because sophisticated hybridisation techniques must be applied to large amounts of tumour cells. In hamster fibroblasts transformed² by herpes simplex virus 2 (HSV-2), viral RNA sequences have been detected³, but not viral DNA; 60% of these transformed cells reacted with a herpesvirus antiserum, using immunofluorescence techniques applied to unfixed cells⁴, but the background of positive cells was 10%, in control cells not treated with HSV-2. To confirm or complement hybridisation and immunofluorescence techniques, alternative methods may be useful.

We have tried to transfer the nucleic acids of a transformed cell line to normal cells, and to check whether functional viral nucleic acid had been transferred, by assaying the recipient cells for herpetic antigen. For the assay, we used immune lymphocytes since we found that the donor transformed cells were susceptible to specific toxic action of lymphocytes from appropriately immunised animals (lymphocytotoxicity test); in addition, killed transformed cells could be used as antigens to stimulate lymphocytes from immune animals (one way mixed cultures). We shall first describe the antigenic characteristics of the transformed cells before reporting the attempts to transfer these properties to normal cells, by means of nucleic acid transfer.

To provide immune lymphocytes for the cytotoxicity test, 4 to 6-week-old hamsters were inoculated subcutaneously with 2×10^6 cells of the 333 line transformed by HSV-2. Tumours appeared 1-2 week later, in 80-100% of the inoculated animals. Four weeks after inoculation, hamsters were killed and a lymphocyte suspension was prepared from the spleen. Toxicity of these lymphocytes was tested⁵ on cells prelabelled with ⁵¹Cr; Table 1 shows that lymphocytes from tumour-bearing animals were the most toxic for the homologous HSV-2 oncogenic cell line but were also active on HSV-2 infected

cells. The effect was low or absent on a normal hamster cell line, as well as on an adenovirus type 12 oncogenic cell line (NilTr.). Conversely, hamsters inoculated intraperitoneally with ultraviolet-irradiated purified HSV-2 virions possessed lymphocytes which were the most toxic for HSV-2 infected cells, but also released ⁵¹Cr from HSV-2 transformed cells and from HSV-1 infected cells, and had no effect on normal hamster cells.

These experiments showed that 333 transformed cells can be used as herpetic antigen targets; to investigate whether they could be used as an antigenic stimulus, we combined two published methods: one showed that mixed lymphocyte cultures can be miniaturised and performed with whole heparinised blood, without purification of the lymphocytes⁶, and the other applied the mixed lymphocyte reaction to the detection of tumour antigens⁷. We inoculated hamsters subcutaneously with 2×10^6 cells from the 333 line and killed some of them at various times after inoculation; their heparinised blood was cultivated for 5 d in the presence of mitomycin C-treated 333 cells. Tritiated thymidine was added on the fourth day, and its incorporation counted on the next day. The index of stimulation was considered positive when lymphocytes incorporated at least three times more thymidine after cultivation in the presence of the antigen than after culture in nutrient medium alone. Immune lymphocytes

Table 1 Toxicity of immune lymphocytes for various target cells

Lymphocytes from hamsters inoculated with	⁵¹ Cr release after action on cells transformed with				Normal (C13)
	HSV-2	Adeno-virus 12	HSV-2	HSV-1	
Transformed HSV-2 cells (333)	64*		12 19	0 8	0
	25	0	3		
Irradiated HSV-2 virions	35		51	23	0

* Specific ⁵¹Cr release (%).

Lymphocyte suspensions were prepared from spleens of hamsters which either bore a subcutaneous tumour resulting from inoculation of 333 cells 4 weeks previously or had been immunised by two intraperitoneal injections of 5×10^5 ultraviolet irradiated HSV-2 plaque-forming units. 10^6 lymphocytes were added to cups containing monolayers of 10^4 target cells, grown for 20 h after labelling with ⁵¹Cr; 18 h after lymphocyte addition, radioactivity released into 100 µl of the supernatant was counted.

showed the highest index of stimulation when collected 1 week after inoculation of the antigen; the index decreased below significant values when the tumour appeared, a confirmation of other observations⁷. Table 2 shows the results obtained with lymphocytes collected one week after inoculation of the antigens. Lymphocytes from hamsters immunised with 2×10^6 HSV-2 transformed cells, or with ultraviolet-irradiated HSV-2 virions, were stimulated specifically by the transformed cells. Hamsters inoculated with 2×10^6 HeLa cells, or with 10^5 - 10^6 normal cells aspirated from the cervix of healthy women, did not react; however, one hamster showed a weak but significant reaction with the 333 cells after inoculation with about 10^4 cells from a secondary culture of a biopsy of human carcinoma of the cervix. The specificity and reproducibility of the latter result must be confirmed. It has been shown recently⁸ that lymphocytes from women with cervical cancer were cytotoxic to HeLa cells, indicating the existence of an antigen common to these two types of cells; our results do not favour the hypothesis that this antigen is herpetic.

Having demonstrated that immune lymphocytes can detect herpes antigens in cells, we then tried to transfer the expression of these antigens to other cells by means of a nucleic acid transfer method used to rescue the SV 40 genome from transformed cells⁹; we chose this method because it was shown to be more efficient than cell fusion¹⁰. Hep-2 cultures were grown

Table 2 Mixed culture of immune lymphocytes with various mitomycin C-treated cells

Blood from hamsters inoculated with	³ H-Thymidine incorporation in the presence of cells		
	Transformed with HSV-2 (333)	Infected with HSV-2	Normal (C 13)
333 cells	36*		8
Irradiated HSV-2 virions	25	54	0
Human cervical carcinoma culture	4.3		1
Normal cervical cells	0.88 ± 0.48†	1.1	
HeLa cells	2.6	2.7	0.5

* Index of stimulation: amount of ³H-thymidine incorporated in the presence of stimulating cells divided by the amount incorporated after culture in nutrient medium alone.

† Mean ± s.e. of six tests performed after inoculation with 10⁵–10⁶ cervical cells from six women.

One week after a single injection of the antigens, hamster blood was collected with 10 U ml⁻¹ of heparin without preservative; aliquots of 0.1 ml of blood were added to 2 ml nutrient medium with 5% inactivated foetal calf serum, containing 4 × 10⁵ stimulating cells pre-treated for 30 min at 37° C with 60 µg ml⁻¹ of mitomycin C. After 4 d culture, 1 µCi ³H-thymidine ml⁻¹ was added and 24 h later the amount of label incorporated into acid-insoluble material was counted.

for 24 h after treatment with nucleic acids from 333 or Hep-2 cells, or from HSV-2 virions; they were then either labelled with ⁵¹Cr and used as targets for spleen lymphocytes from hamsters immunised with HSV-2 grown in Hep-2 cells; or they were treated with mitomycin C and used as stimulating antigens in mixed cultures with heparinised blood from the same immunised hamsters. In both tests, lymphocytes immune to HSV-2 reacted with Hep-2 cells treated with nucleic acids from the transformed cells, but not with untreated Hep-2 cells (Table 3). The immune lymphocytes also reacted with Hep-2 cells infected with either HSV-2 virions or HSV-2 DNA; the latter result is not unexpected since the experiment was done under the conditions used to demonstrate that HSV DNA is infectious^{12–14}. After treatment with nucleic acids from the transformed 333 cells, there was a zone phenomenon, since Hep-2 cells treated with 0.1 µg nucleic acids were more responsive than those treated with 5 µg. A nucleic acid transfer experiment was then repeated, using hamster C 13 cells as recipients; the appearance of herpetic antigens in these cells

Table 3 Reaction of lymphocytes immune to HSV-2 with Hep-2 cells grown after treatment with various nucleic acids (NA).

Hep-2 cells grown with	Used as	
	Target cells for lymphocytotoxicity	Stimulating cells in mixed culture
Exp. 1		
PBS	0*	
Hep-2 NA 5 µg	0	
0.1 µg	0	
333 NA 5 µg	6	
0.1 µg	9	
HSV-2 NA 5 µg	69	
0.1 µg	10	
HSV-2 virions	52	
Exp. 2		
PBS	0	1†
333 NA 5 µg	2	16
0.1 µg	32	55.5
HSV-2 NA 5 µg	29	51
0.1 µg	3	6.5

* % ⁵¹Cr release from Hep-2 cells.

† Stimulation index of thymidine incorporation into lymphocytes from hamsters immunised with ultraviolet HSV-2.

After extraction¹¹ from 333 and Hep-2 cells and from HSV-2 virions, nucleic acid solutions at 5 and 0.1 µg ml⁻¹ were prepared and mixed with CaCl₂ at final concentration of 125 mM (ref. 12) before inoculation of 0.5 ml nucleic acid solution to two Petri dishes containing 10⁶ cells each. After 20 min at room temperature, each dish received 5 ml of nutrient medium and the cells were grown for 24 h at 37° C. They were then washed, suspended, and ⁵¹Cr labelled for lymphocytotoxicity test (see Table 1) or treated with mitomycin C for the mixed culture test (see Table 2).

could also be demonstrated (results not shown).

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Athymic (nude) mice express gene for myxovirus resistance

DISAPPOINTINGLY little is known about the mechanisms of natural, genetically determined resistance to infectious agents¹. Even in the simplest situation, where a single, dominant gene is responsible for resistance to a group of closely related viruses, no satisfactory explanation of the phenomenon has been offered. A case in point is the resistance towards the lethal action of a number of myxoviruses, mainly influenza A, exhibited by the inbred mouse strain A2G and attributed to the presence of the dominant gene *Mx* (refs 2–5). Serial virus titrations in A2G and comparable susceptible mice showed that resistance must depend on an event occurring early in the infectious process, since by day 2 after challenge virus titres were significantly lower in resistant animals². It was conceivable that an early triggering of the immune system might exert such an effect. If this were the case, one would expect the expression of the resistance gene to be impaired in mice showing profound disturbances of their immunological apparatus. Nude mice homozygous for the gene *nu* (refs 6–9) show such a disturbance, mainly of the T cell system. We have therefore introduced the gene *Mx* into *nu/nu* mice, and we have investigated the progeny from appropriate crosses for the phenotypic expression of resistance to neurotropic influenza virus.

Nude males (genotype *nu/nu* on a predominantly BALB/c genetic background, from G.L. Bomholtgard, Ry, Denmark) were mated with A2G females (*Mx/Mx*, bred locally). The F₁ generation was normal with respect to coat and thymus development, and was resistant to intracerebral challenge with 100 LD₅₀ (as estimated in susceptible mice) of neurotropic influenza A₀ virus, strain NWS (ref. 3). Brother-sister matings

Table 1 Resistance of nude F_2 mice* to neurotropic influenza A virus

Experiment No.	No. challenged	Resistant†	Susceptible‡
1	9	8	1
2	11	8	3
3	11	8	3
Total	31	24	7

* 75% expected to be Mx carriers.

† Survived intracerebral challenge with 100 LD₅₀ of stock NWS virus for 14 d or longer.

‡ Died of viral infection within 5–7 d.

among F_1 mice yielded an F_2 generation, of which approximately 25% were phenotypically nude. Assuming independent segregation of the genes nu and Mx , 75% of these nude F_2 should carry the gene Mx either in homozygous or heterozygous form. At the age of 3 weeks, nude F_2 together with comparable numbers of non-nude littermates, and with control resistant (Mx +) and control susceptible mice for monitoring the challenge virus, were inoculated intracerebrally with 100 LD₅₀ of NWS. All susceptible control animals succumbed to viral infection, whereas all control resistant Mx +/+ mice survived. Of both nude and non-nude F_2 mice approximately 75% survived. The results of the nude group are shown in Table 1; the observed seven deaths among 31 challenged mice are close to the expected 25%.

It is thus apparent that the nude phenotype did not preclude expression of virus resistance. The numbers observed also

antigen of influenza A virus. Tests on delayed hypersensitivity were not performed.

Expression of resistance in Mx -bearing mice seems therefore independent not only of a functional T-cell system, but also of the orderly development of HAI antibodies, usually regarded as the protective antibody in influenza virus infection. These findings agree with the observations that resistance of A2G mice could not be broken by various immunosuppressive regimens, including whole body X irradiation and neonatal thymectomy (P. A. Klein, personal communication, and unpublished work).

We conclude that the inborn resistance of mice carrying the dominant gene Mx does not require an intact immunological machinery for its expression. Both cellular and humoral immunity may be less important in recovery from influenza virus infection than hitherto suspected.

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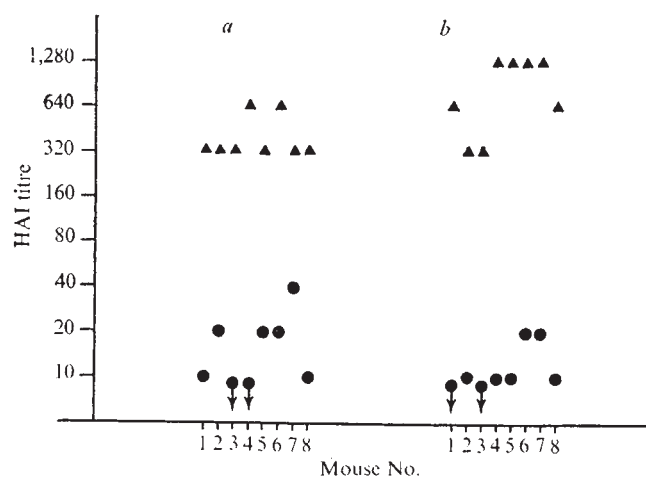


Fig. 1 Serum antibody titres in myxovirus-resistant athymic and control mice surviving 100 LD₅₀ of influenza A NWS. ●, Congenitally athymic (nude) mice; ▲, non-nude littermates. a, Serum taken on day 14; b, serum taken on day 21.

suggest that the genes Mx and nu are not closely linked. To make sure that those mice which were assumed to be nude on the basis of their coat development also had abnormal thymuses, a few survivors were examined histologically. We found the typical picture of thymic dysplasia with cystic and tubular structures among irregularly arranged epithelial-like cells surrounded by connective tissue. Spleen and lymph nodes were found depleted of lymphoid cells in the thymus-dependent areas^{9,10}. To measure functional impairment of the T cell system in nude, Mx -bearing mice, use was made of the fact that formation of haemagglutination-inhibiting (HAI) antibodies is a T cell dependent function¹². Two and three weeks after virus challenge, individual surviving nude and non-nude littermates were bled and the titres of HAI were determined. Figure 1 shows the results: nude mice surviving virus challenge had significantly lower antibody titres than similar non-nude littermates. The same difference was also revealed with antibodies to the complement-fixing nucleoprotein

Location of nuclear proteins on the chromosomes of newt oocytes

In eukaryote cells chromosomal proteins are responsible for the organisational state of the DNA and the control of genetic expression, therefore knowledge of their location is an essential prerequisite for understanding chromosome function. Cytological localisation of chromosomal proteins is feasible using cells with giant chromosomes such as the oocytes of the newt *Triturus cristatus carnifex* where there are clearly defined regions active in RNA transcription (the loops) and regions containing most (>95%) of the DNA in a condensed state (the chromomeres)¹. There are two main classes of protein associated with chromosomes: histones, which are generally considered to be complexed with the sugar-phosphate backbone of the DNA²; and nonbasic proteins which are constituents of metabolically active chromatin³.

We extracted the nonbasic proteins from ribonucleoprotein (RNP) particles which are released from the chromosomes into the nucleoplasm⁴. These nonbasic proteins have been shown previously⁵ to comprise a heterogeneous size distribution of polypeptides. Here the polypeptides were separated on a preparative scale by column chromatography and injected into rabbits to stimulate antibody production. Histone protein was extracted from newt liver chromatin and was also used to produce antibodies. By treating chromosome preparations with

fluorescein-conjugated antibodies the chromosomal sites of the various proteins were determined.

Nuclear RNP particles were isolated from homogenates of newt oocytes as described previously^{4,5}. The final step in purification was the collection of the peak fraction from a 30–50% (w/v) sucrose gradient. At all stages in isolation, solutions contained 5 mM 2-mercaptoethanol to prevent protein aggregation⁶. The RNP was solubilised in SDS-buffer (0.1% sodium dodecyl sulphate; 10 mM Tris-HCl, pH 7.4; 10 mM 2-mercaptoethanol; 50 mM NaCl) and dialysed for 4 h against

fraction was analysed by SDS-acrylamide electrophoresis. These were compared with electropherograms of total RNP protein and of total histone (Fig. 2). Of the 13 main bands in the total RNP extract, between two and four bands were contained in each of the five gel-filtration fractions and distributed between these fractions in groups of decreasing molecular weight (see also Table 1). The separation of the proteins by gel filtration was judged to be satisfactory.

As antigen sources, 0.5 ml samples of total RNP protein, fractionated RNP proteins and total histone were each incorporated into multiple emulsions⁷ and injected into different rabbits. Similar samples were injected into different rabbits on three separate occasions to eliminate any anomalous effect due to a particular animal. After a minimum of 60 d the rabbits were bled and the sera were prepared as described previously⁸.

The various antisera were reacted with chromosomes as follows. Lampbrush chromosomes were prepared from newt oocyte nuclei⁹ and firmly attached to the surfaces of coverslips by centrifugation through Tris-buffered saline⁴, at pH 7.8, to obtain maximum transmission of fluorescence. The chromosome preparations were treated with various dilutions of antisera in buffered saline for 30 min, washed in buffer for 1 h, placed in fluorescein-conjugated sheep anti-rabbit antibody solution (Wellcome Reagents Ltd) at a dilution of 1:100 and again washed

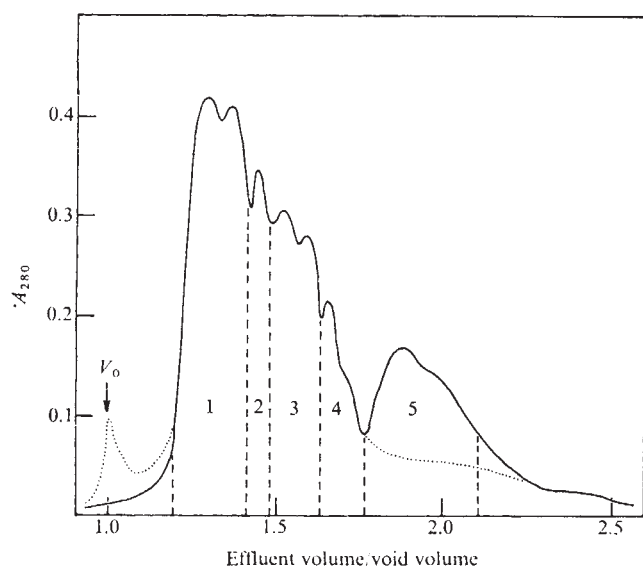


Fig. 1 Separation of nuclear RNP proteins by column chromatography. An 0.5 ml solution of SDS-solubilised protein was applied to a 56 cm column of Sephadex G-200 Superfine gel and eluted with SDS-buffer. The eluted material was divided into five fractions as shown on the A_{280} profile. At the void volume of the column (V_0) there was occasionally elution of contaminating RNA. The peak assigned as fraction 5 was not present in all preparations (dotted line).

this buffer. The solubilised RNP complex was centrifuged at 95,000g for 16 h to remove RNA by pelleting. To separate the RNP proteins on a molecular weight basis a 0.5 ml sample was eluted through a column of Sephadex G-200 gel with SDS-buffer. The elution profile of absorbance at 280 nm is shown in Fig. 1. Resolution of peaks was not as good as was obtained by SDS-acrylamide gel electrophoresis but large amounts of protein could easily be separated. The eluted protein was divided into five fractions each of which was concentrated to 0.5 ml by pressure dialysis. The complexity of protein content in each

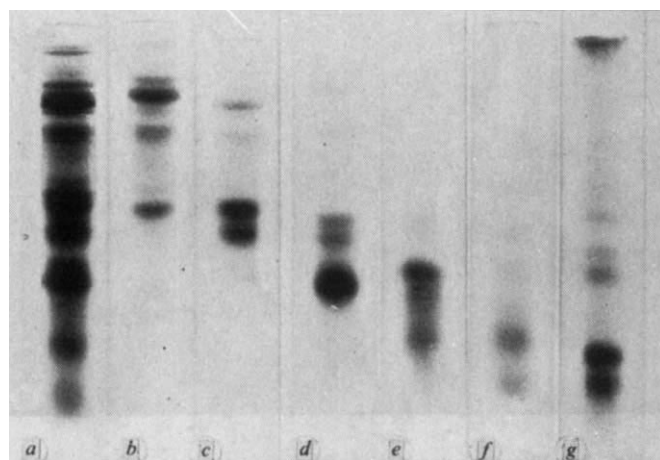


Fig. 2 SDS-acrylamide gel electrophoresis of solubilised proteins. *a*, Total nuclear RNP protein; *b–f*, fractions 1–5 derived by gel filtration (Fig. 1); *g*, newt liver histones (kindly supplied by Dr D. B. Malcolm). 20 μ l samples were applied to 7% polyacrylamide gels containing 0.1% SDS and 0.1 M phosphate buffer, pH 7.1. Electrophoresis was at 10 mA per tube until the buffer front had migrated 6–7 cm. The gels were fixed and stained with Coomassie blue.

Table 1 Characteristics of antisera produced against chromosomal proteins

Antiserum produced against	Molecular weights of main components used as antigen	Titre (greatest dilution giving a fluorescent reaction)	Specificity of reaction with lampbrush chromosomes
Total nonbasic protein	155,000–17,000 (13 bands)	1 : 2,500	With all loops and with chromomeres to a lesser extent
Fraction 1	135,000 125,000 90,000 50,000	1 : 1,750	With all loops
Fraction 2	65,000 50,000	1 : 1,750	With all loops
Fraction 3	65,000 50,000 35,000	1 : 2,500	With only 10 pairs of loops
Fraction 4	35,000 20,000	1 : 1,750	With all loops and with chromomeres to a lesser extent
Fraction 5	20,000 17,000	1 : 1,500	Mainly with chromomeres
Total histone	20,000 16,000	1 : 2,500	With chromomeres
Normal serum	—	1 : 500	With all structures

in buffer. The chromosomes were located by phase contrast microscopy and then the fluorescent reaction was observed in a beam of ultraviolet light using the appropriate exciter and barrier filters.

Antiserum produced against the extract of nonbasic proteins reacted specifically with the chromosomes and more intensely with the loops than with the chromomeres. The antiserum produced against histone reacted primarily with the chromomeres (Fig. 3d). Control preparations using normal rabbit serum reacted nonspecifically with all structures, nucleoli as well as chromosomes, but only at a relatively high serum concentration. The greatest serum dilution at which a fluorescent reaction could be obtained (see Table 1) was routinely used to avoid cross reaction and nonspecific effects. There was some reaction with the loops with anti-histone serum but this is most likely to result from contamination of histone extract with some nonbasic protein. In fact a few minor bands of high molecular weight protein could be seen on the electropherogram (Fig. 2). Low concentrations of histones may well be present in the loops but the technique used here is probably not sensitive enough to detect these.

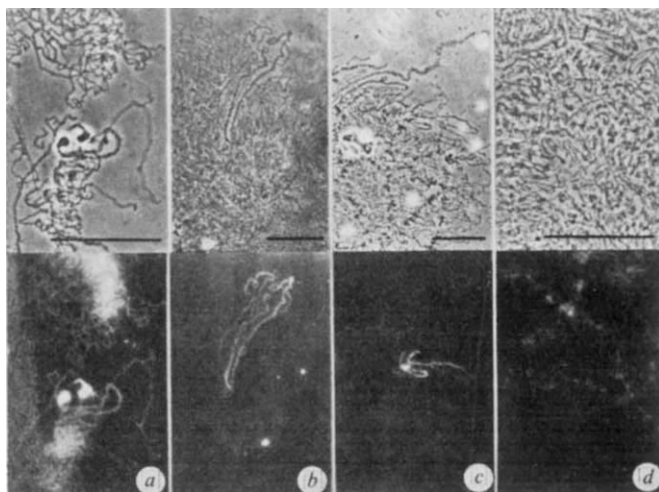


Fig. 3 Phase contrast and fluorescence photomicrographs showing location and specificity of reaction of fluorescein-conjugated antibodies with lampbrush chromosomes. *a*, Antiserum produced against RNP-protein fraction 2 at a concentration of 1:1,000; *b* and *c*, antiserum against fraction 3 at a concentration of 1:1,250; *d*, antiserum against histone at a concentration of 1:1,000. The photomicrographs were taken on Ilford FP4 (1 s exposure for phase; 2.5 min exposure for fluorescence) and developed in Paterson's Acutol. The bars represent 10 μ m.

Of the protein fractions derived from solubilised RNP-fractions 1, 2 and 4 stimulated antibodies which reacted specifically with all the chromosome loops (Fig. 3a), that is, one or more of the proteins present in these fractions were common to the RNP matrix of all loops. As a result of the distribution of different polypeptides between these fractions (see Fig. 1 and Table 1) at least two different proteins are common to all loops. Antibodies produced against fraction 4 showed some cross reaction with the chromomeres. Furthermore, antibodies against fraction 5 reacted specifically with the chromomeric axis. Fraction 5 gave a very similar banding pattern on SDS-acrylamide gels to that of histone (Fig. 2). Because this fraction was not present in all RNP protein extracts (Fig. 1) fraction 5 was judged to be contaminating histone probably as a result of excessive homogenisation of the oocytes.

The most interesting and unexpected result was given by antiserum produced against fraction 3. This antiserum reacted with the proteins associated with only certain loops—approximately 10 loop pairs in each chromosome complement. These loops did not have any peculiar morphology and could not be

identified before the fluorescent reaction (Fig. 3b and c). As far as can be discerned the same loops give this specific reaction in all chromosome preparations and are randomly distributed. Because the whole length of spatially isolated loops gives a specific reaction this is conclusive evidence of the integrity of the loop structure, that is, the lampbrush loop is a functional unit. Evidence that the loop is a unit of transcription will be reported later (J.S., unpublished work). The protein which is the main component of fraction 3 and which probably elicits the specific loop reaction has a molecular weight of approximately 35,000.

Thus histones are present primarily in the regions of condensed DNA (chromomeres) in lampbrush chromosomes (previously shown to contain basic protein by staining¹⁰ while RNP-derived nonbasic proteins are restricted to the loops. The implications of the high protein content of nuclear RNP remains unknown. Preliminary evidence suggests that various types of enzymic activity are present, specifically the ability to polymerise ribonucleotide triphosphates and to cleave large nuclear RNA into smaller fragments. Homoribonucleotide polymerases¹¹ and endonuclease¹² have already been reported to be present in mammalian RNP particles. Obviously enzymes concerned with the processing of primary transcript RNA can only account for a very small amount of protein which may even not be visible in gel electropherograms. A secondary RNP formed during oocyte maturation has been isolated and has been shown to have a similar protein constitution to the RNP described here. Results pertaining to the protein content of the different RNP products at various stages in the processing of RNA transcripts will be reported later.

We thank Dr D. B. Malcolm for supplying the histones and Professor H. G. Callan for critically reading the manuscript.

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Erratum

In the article "Deterioration of high school students' attitudes to physics" by P. L. Gardner (*Nature*, **250**, 465; 1974) the following corrections should be made to the 3rd paragraph: line 5, for 4 yr read 2 yr; lines 10-11 should read "in coeducational State high schools in moderately affluent suburban areas of Melbourne."

matters arising

Geomorphological dating of cave openings in South Africa

IN a recent article in *Nature*¹ Partridge dates South African hominid sites with remarkable precision by relating them to retreat of cyclic nickpoints. The soundness of his analysis and results depends on the validity of his geomorphological framework, the date of 20 Myr assigned to the inception of his cyclic nickpoints and his method.

The framework is well known and provides for the existence of identifiable remnants of 'Gondwana', 'post-Gondwana', 'African' and later cyclic surfaces of former continental extent². The dating and correlation of erosion surfaces over wide areas of Africa with quite different climatic and tectonic histories rest, however, on shaky grounds³. Moreover, results of recent work connected with the South African oil search on land and offshore contradict the framework in many material respects. Lack of space makes it impossible for me to elaborate here, but, as an example, the Paleogene, during which the African surface on land reputedly stood so low that the rivers were sediment starved⁴, saw the most rapid deposition off the Natal coast from the mid-Cretaceous onwards.

The dating of 20 Myr for the inception of the 'Post African 1' cyclic nickpoint, based on transgressive sediments above an unconformity at Uloa, Natal⁵, is taken as factual. The correlation of transgressive coastal sequences with incision on land is debatable, as it presupposes rather special conditions: but if we accept that the sediments above the unconformity could record the first incision of the 'African' surface, the figure may have to be doubled: in the J (c)—1 well, drilled 24 km off Stanger, the unconformity and 'Pecten Bed' are clearly identifiable, but the age is early Oligocene⁶. Relationships at Uloa, on which so much has been based, are representative of one stand of a slowly migrating shoreline.

Briefly, Partridge's method involves the calculation of mean rates of nickpoint recession in respect of each site, which are then plotted in relation to the midpoints of the segments affected by incision. He assumes that nickpoint migration was subject to linear decline culminating in zero at the stream sources, and apparently reads off the rates of migration at the hominid sites from the graph in Fig. 2. He admits

that the assumption is erroneous, but maintains that the errors of the linear interpolation technique are greatly reduced due to the close proximity of the sites to the headwaters. He thus makes an assumption that seems to be basic to his argument and calculations, admits that it is erroneous, but brushes away any possible reservations by implying that migration rates near the sites were so low that they would have been little affected by what happened downstream anyway.

Recognition of small nickpoints so far upstream as expressions of an ancient coastal event cannot be an easy exercise in a region with numerous nickpoints clearly attributable to differential rock resistance, warping, and so forth. Moreover, a method based on nickpoint recession cannot be applicable to a major basin such as the Orange, where great differences in gradient and channel characteristics reflect the extreme variations in lithology. The present regime illustrates the influence of climate: below Prieska the flow probably decreases at normal times downstream, and nickpoint recession rates would presumably not have decreased upstream under comparable past climates. My gravest criticism, however, is that the implications of differential nickpoint recession up a main stream and its tributaries are ignored. Thus the Orange, the Vaal, the Harts and a small tributary are lumped together, although they are clearly not amenable to treatment as a single unit; this procedure presupposes that recession rates are mainly dependent on distances of watersheds and nickpoints from the main drainage outlet to the ocean, and not on conditions along the stream courses themselves.

Partridge's methods and basic assumptions are of doubtful scientific validity, and his datings can only serve to confuse and mislead South African anthropologists.

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Received January 22; revised April 1, 1974.

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DR PARTRIDGE REPLIES: Dr de Swardt's comments, although relevant to the technique and results reported in my article, reflect fundamental misconceptions as well as lack of familiarity with recent geomorphological research in South Africa. It would therefore be unprofitable to take issue with him point for point, though I should like to refute certain statements of his which have direct bearing on my argument.

(1) I claimed no "remarkable precision" for my estimates for the date of first cave opening at the various hominid sites. My results were presented cautiously and with deference to limitations in accuracy imposed by availability of data for the construction of the nickpoint migration graphs, and the resulting possibility of small scale fluctuations between observation points.

(2) The geomorphological framework which de Swardt calls to question is firmly based on scientific observation and measurement, and does not rely on the unsupported views of any one worker. In particular, Professor L. C. King has never claimed that all of the cyclic surfaces referred to by de Swardt were of former continental extent. This is a totally unreasonable proposition, and was certainly not applied in the context of my work. Indeed, the study of such surfaces in southern Africa has revealed that some are represented over limited areas, others have been partially planed and most have been subject to post-formational warping. All these factors have been subjected to detailed analysis (see references in my original article), and the results have been taken into account in the application of my technique.

(3) There is no contradiction of the geomorphological framework which I applied in the records of offshore oil prospecting cited by de Swardt. Various workers, including King, have recorded sedimentation of the continental shelf, dating from mid-Cretaceous to early Tertiary times¹⁻³, which can

be referred to onshore incision in the African erosion cycle. These offshore results merely add to the overwhelming body of evidence in support of the framework which de Swardt wishes to discredit.

(4) In assessing the date of inception of the Post-African I erosion cycle, the age of littoral sediments immediately overlying the Post-African I planation surface must be conclusive. Where such sediments have been preserved, not only at Uloa, but along considerable stretches of the South African coast between East London and Saldanha Bay, their age has proved to be Miocene³. Simple palaeontological principles do not permit that the Oligocene "Pecten Bed" identified 24 km offshore during drilling operations is the same biostratigraphical unit as that of Uloa, since both the shark and Pecten fauna at the latter locality have been confidently placed within the Miocene on the basis of reliable evidence^{4,5}. The 20 Myr date for the inception of the Post-African I incision can therefore be accepted with confidence. Relationships along considerable stretches of the coast do not indicate a slowly migrating shoreline, but several phases of transgression during the Tertiary^{3,6}.

(5) A small margin of error in my assessment of nickpoint migration rates cannot be avoided, as the linear assumption provides for a mean representation, which cannot readily be refined, as the affects of some variables are impossible to measure. This is a potential source of some inaccuracy, but can by no stretch of argument be regarded as fatal to the technique. The absolute range of possible inaccuracies must, of necessity, decrease towards the watershed origin. This range is considered to be sufficiently small to permit the technique to be used to derive general orders of age and a relative sequence of dates for cave opening—all that I originally claimed for my results.

(6) Numerous erosional nickpoints in South Africa can be recognised through careful fieldwork. Their cyclic associations are not difficult to determine through the application of careful morphometric and statistical analyses such as formed the basis for my article. Erosional nickpoints are clearly distinguishable from those of lithological or local tectonic origin.

(7) Decline in nickpoint migration rates upstream is by no means exclusively a function of diminishing discharge. Owing to large scale Plio-Pleistocene upwarping of the South African coastal margins, many rivers show substantially steeper gradients along their lower courses than in higher reaches; for example, between the Orange River mouth and Prieska,

the mean channel gradient is 0.9 m km⁻¹; between Prieska and Buxton on the Harts River the gradient is 0.4 m km⁻¹.

(8) Variations in nickpoint migration rates between trunk and tributary channels are, indeed, taken into account in my analysis, since my measurements are related to the actual channel segments concerned. Varying conditions along such segments have naturally affected the present positions of the nickpoints which have migrated along them, upon which my graphs are based. The limited inaccuracies which may result from small-scale fluctuations between observation points have been considered.

(9) The validity of my method and assumptions is further confirmed by two age estimates based on entirely different techniques. My estimate of 4.8 Myr for the 61 m terrace of the Vaal River at Windsorton⁷, based on the same methods used to estimate dates of first cave opening, is generally confirmed by the presence at this locality of elephant remains which existed some 4 Myr ago in the East African potassium-argon chronology⁸. Moreover, Butzer⁹, in a lengthy analysis to be published in December, has independently arrived at a maximum age estimate almost identical to my own for the Taung hominid deposit.

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Ascorbic acid and nitrosamine

SIR—Edgar¹ has proposed that ascorbic acid might inhibit the carcinogenic action of nitrosamines and other carcinogens that may act by alkylation. The rationale was that ascorbate might be alkylated *in vivo* before the carcinogens can react with cell macromolecules. The experimental basis for Edgar's thesis was that statement by Kamm *et al.*² that ascorbate inhibited the liver necrosis induced by dimethyl-

nitrosamine (DMN). This statement, however, was presented without experimental details and was subsequently withdrawn³.

The main concern of the study by Kamm *et al.* was the inhibition by ascorbate of the liver toxicity induced by oral administration of aminopyrine plus nitrite. This study followed our report in 1972 suggesting, on the basis of *in vitro* experiments, that ascorbate might be used to block *in vivo* formation of N-nitroso compounds from nitrosatable chemicals (for example, drugs), since ascorbate efficiently reduces nitrate⁴. Subsequently, the report of Kamm *et al.*² appeared. Greenblatt⁵ found similar results in mice to those of Kamm *et al.*, but stated that ascorbate did not affect DMN toxicity. We found that ascorbate prevented liver damage from gavage of dimethylamine plus nitrate to rats and, from experiments presented in detail, that ascorbate did not significantly affect the production by DMN of liver necrosis and elevated serum transaminase levels⁶. Ascorbate did not affect transplacental carcinogenesis in rats by ethylnitrosourea, but inhibited carcinogenesis by ethylurea plus nitrite⁷.

We are concerned that our original suggestion should not be extended without basis to the hypothesis that ascorbate might have a much wider inhibitory action on carcinogens. The interesting suggestion of Edgar is not supported by the results reviewed here, which were mostly made public after Edgar's paper was submitted.

Yours faithfully,

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reviews

Immunoglobulins

The Antigens. Vol. 1. Edited by Michael Sela. Pp. xiii+573. (Academic: New York and London, January 1974.) \$31; £14.90.

THE appearance of a multivolume work about any topic is a sign that the subject has now become part of the body of accepted scientific knowledge. A period of rapid progress is coming to an end and there is a sufficient volume of fact and theory to form the basis of undergraduate courses as well as to be an essential part of the background of all scientists working in related fields. In biochemistry, proteins were the first subject to receive this accolade some twenty years ago followed quickly by enzymes, nucleic acids and many others. The aim was to present research workers, teachers and advanced students with a rather full account of the field and on the whole they have served a useful purpose, though of necessity they have been a year or two out of date by the time they reached the libraries. Now it is the turn of immunochemistry, or as some would have it, molecular immunology. How does it match up?

The first glance is disconcerting as volume 1 of *The Antigens* contains four chapters on antibodies, one general chapter on protein evolution and only two on antigens. Volume 2, of which the contents are listed, will have three chapters, each on antibodies and antigens. This is perhaps not important, as any potential reader will be well aware that it is hardly possible to write of antigens without extensive reference to antibodies and indeed much of the progress in knowledge of antibody structure, genetics and synthesis has come from study of their antigenic properties, but it would have been helpful if the title had given a better guide to the contents.

The antigens chosen for discussion in this volume are nucleic acids by Dr David Stoller and enzymes by Dr Ruth Arnon. The former are difficult to handle as injection of purified nucleic acids into an animal of different species rarely gives rise to a significant immune response and specific antibodies are found only when protein-nucleic acid complexes are used; that is the nucleic acids are behaving as large molecular weight haptens. Nevertheless, in recent years satisfactory antisera have been obtained (and have been found to

occur spontaneously in pathological conditions) and have been used effectively in investigations of the structure and function of nucleic acids in cell and subcellular fractions and a useful summary of the work is given here. Enzymes are much more easy to handle as antigens and Dr Arnon has led much of the work directed to using specific antisera to gain information about their structure and catalytic activity. This has proved a valuable adjunct to the very extensive studies of enzymes by protein chemists, X-ray crystallographers, enzymologists and all the others and, over an increasing range, has complemented and confirmed knowledge of these key molecules.

Immunoglobulins are the major subject of this book, about a quarter of which is given to their structure, and rather less space given in turn to the immunoglobulin allotypes, phylogeny of immunoglobulin and the chemistry and biology of immunoglobulin E. Dr Gall has given a good account of the chemical structure which is now well understood, but it is unfortunate that the article was written just too early to include the recent work of the X-ray crystallographers which is clarifying the nature of the combining site and other topics which he discusses. Allotypes have long had a fascination for some of us, particularly the *a* locus allotypes of the rabbit which remain the odd man out in most of the work on these allelic variants of the immunoglobulins. These investigations have contributed greatly to knowledge of their genetic origin and the conviction remains that when their behaviour is fully understood it will give a much clearer understanding of the source of the multiplicity of form of immunoglobulins. This chapter by Dr Rose Mage and colleagues has got all the facts and some of the speculation well presented. Dr Ishizaka has given perhaps the most personal account in his chapter on IgE, the minor immunoglobulin class responsible for immediate type hypersensitivity and apparently of major importance in the immune defence of the host against a variety of parasites. Of obvious clinical importance, this is a field where the myeloma proteins, for a long time the only source of large amounts of homogeneous immunoglobulin, have been of exceptional importance.

A general chapter on protein evolution by Dr Norman Arnheim serves as an introduction to the phylogeny of

immunoglobulins by Drs Kubo, Zimмерman and Grey. This is another way to try and understand the genetic origin of the complexity of immunoglobulin structure and, although the contributions from this approach have not so far been of major importance, it is well worthwhile from this point of view as well as for its intrinsic interest in biology. The transition from invertebrates to vertebrates seems to have been the most important point in the development of a specific immune response and much of the work described centres on the immunoglobulins of the lower vertebrates. No evidence has, however, been found so far for the original gene product of 11-12,000 molecular weight from which the immunoglobulins of present vertebrates are believed to have been derived by gene duplication.

The chapters are reasonably balanced in content, but their arrangement seems arbitrary. Together with the second volume a fair coverage is given of the more biochemical aspects of immunology, though there are surprising omissions such as the absence of any discussion of immunoglobulin biosynthesis. Perhaps with the title of 'The Antigens' this is not surprising, but with more than half the space given to immunoglobulins, biosynthesis would seem a stronger candidate for inclusion than, say, the individual classes of immunoglobulin. The editor's difficulties are apparent and it is perhaps questionable whether these multivolume works are the most effective way of summarising present information. The alternative of an advanced textbook giving a briefer but more integrated survey and supplemented, for the enthusiast, by the annual volumes of reviews on specialised topics has much to commend it. There is no doubt room for both and this volume and its successor should be a useful source of reference for some time to come.

R. R. PORTER

Betas and muons

Beta Decay and Muon Capture. By Masato Morita. Pp. xi+361. (Benjamin: Reading, Massachusetts and London, March 1974.) \$19.50.

THIS is a curious book, though it will probably be very useful to a specialised audience. It has grown from a course of postgraduate lectures given at the University of Osaka. Judging by the

notation and the balance of the material, the course was written in the early sixties and has not changed its shape since then. The first five chapters, which occupy half of the main text, give an introduction to weak interactions. There is a great deal of historical detail on the Fermi and Gamow-Teller theories. Parity non-conservation is treated at length, as if it were a brand new phenomenon, before a long and circumstantial chapter on the V-A theory. The weak interactions of elementary particles are given a reasonable amount of space, but with an alarming vagueness. This is certainly not the place to read about the Cabibbo theory, for instance. Its results are mentioned, but after a page and a half of introduction the author announces that "we do not have space to introduce the SU3 computation". Surely he could introduce Clebsch-Gordan coefficients, by analogy with SU2, to justify the precise numerical predictions of the theory which he tabulates.

The second half of the main text is devoted to the chief business of the book, a review of beta decay and muon capture as tools for nuclear structure physics. The presentation is pedestrian, but the details of individual experiments are given at unusual length. Although most of the reference lists stop in about 1969, there is a chapter which deals with more recent developments, such as the investigation of second class currents in the transitions of mirror nuclei.

Because of the detail in the later chapters, and because of the patiently long-winded explanations of many technical points, this book should be very useful to experimental students of nuclear physics. But they should come to it after a simple course on the weak interactions of elementary particles, based for instance on some of the excellent reports published by CERN, Geneva (Cabibbo and Veltman: CERN 65-30; and J. S. Bell: CERN 72-4).

D. J. MILLER

Crust of the Earth

Structural Geomorphology. By J. Tricart. Translated by S. H. Beaver and E. Derbyshire. Pp. xiii+305. (Geographies for Advanced Study.) (Longman: London, April 1974.) £4.75.

PROFESSOR TRICART'S book, published in France in 1968, has been admirably translated by two geographers from the University of Keele. It is intended not only for students taking a first degree in geography but also for specialists in the "cognate disciplines" of geology, pedology, ecology and planning. The

book is built round the premise, stated on the first page of the introduction, that the surface features of the lithosphere have been moulded by the interaction of internal and external forces at work in the Earth's crust and the author recognises a close relationship between characteristic landforms and large-scale crustal structures. The first chapter deals with the distinctions between continents and oceans, the second with geosynclines and fold belts, the third with platform areas and the final chapter with faults and volcanoes. Stress is laid on the fact that landscapes evolve over long periods.

This scheme of treatment, together with Professor Tricart's excellent aphorism (page 22) that "nature herself is a unified whole" raises the hope that the book, by bringing geological and geographical thinking to bear on a subject involving both disciplines, may illuminate the whole field of geomorphology. Where details are concerned, the book does indeed provide many clear and well illustrated examples of the relationship between structure and landform.

Further reading, however, raises serious doubts about the geological treatment. The author's views of crustal evolution have much in common with those of Soviet geologists who discount the possibility that large-scale horizontal displacements of continents have taken place during the past few hundred million years. This view is seldom expressed today in English or American works and a reasoned exposition of it might have been welcome. But it is disconcerting to find no discussion of the evidence concerning seafloor spreading (the mid-Atlantic ridge is referred to only once, on page 36, as a structure "somewhat similar to an island arc") and even more so to find on page 45 the statement that "despite the precision of the techniques, no measurements yet made have shown the slightest signs of change in the relative positions of Europe and North America, which should have occurred if the two continents are drifting apart".

This statement, made without any qualification, is the more surprising in that classic palaeomagnetic studies by Runcorn, Tarling and others are cited in the bibliography. Errors of fact are not uncommon: for example, the Moho, though defined as the base of the crust on page 41, is referred to on the next page as a discontinuity within the crust: the *schistes lustrés* are described as a flysch facies. More important, perhaps, from a student's point of view is the lack of a stratigraphical table or any other data from which he can discover the relative ages of the geological systems mentioned, the absence of any account of landforms associated

with depositional processes, and the cursory treatment meted out to rift valleys. Since the geological aspect is fundamental to the author's approach, I feel that defects of this kind undermine the whole structure of the book.

J. WATSON

Excited molecules

Excited States. Vol. 1 Edited by Edward C. Lim. Pp. xii+347. (Academic: New York and London, January 1974.) \$24.50; £11.75.

INTEREST in the structure of electronically excited species in the gas phase has been growing apace in the last two or three decades. A discussion of the Faraday Society in 1963, on just that subject, was an undoubted success. Somewhat earlier (1955) had come a text by Laidler on *The Chemical Kinetics of Excited States*, and Reid's book (1957), *Excited States in Chemistry and Biology*, was a stimulating early work.

The present volume, it might be argued, is not one but six. Certainly, its six chapters differ considerably in subject matter. The first, by G. Wile Robinson, deals with radiationless transitions, whereas the remaining five deal with topics that involve emission or absorption of radiation. Robinson's chapter is much the most readable of the six. The second chapter, by M. A. El-Sayed, contains a comprehensive review of phosphorescence microwave double resonance spectroscopy (PMDR). It seems from this article that there will be a considerable increase in the number of abbreviations that infest the literature.

The third chapter, by Robin M. Hochstrasser and Paras N. Prasad, deals with optical spectra and relaxation in molecular solids. It outlines various aspects of the electronic spectra of solids (molecular crystals and mineral crystals) and contains detailed discussion on phonon interactions that have not previously been treated in detail. The fourth chapter, by Wolfgang Liplay, is an excellent account of the determination of dipole moments and polarisabilities of molecules in excited electronic states. The fifth chapter, by C. J. Selisker, O. S. Khalil and S. P. McGlynn, treats the luminescence of polar aromatic molecules; and the sixth, by A. J. Duben, L. Goodman and M. Koyanagi, deals with interstate interactions in aromatic aldehydes and ketones.

The book is very well produced. I found perhaps half-a-dozen printer's errors, none of them of great import. Further volumes are planned.

A. D. WALSH

Fact and language

Linguistics and Information Science. By Karen Sparck Jones and Martin Kay. Pp. xii+244. (Library and Information Science Series.) (Academic: New York and London, January 1974.) \$14.50; £6.80.

THIS book is a state-of-the-art survey on the use of linguistic theories and techniques in information retrieval. The emphasis is on automated methods; that is, on linguistic theories that have been (or could be) used to develop computational methods of analysing language, and on their use in automated information retrieval systems. The survey concentrates on the literature of 1965–1970.

The authors first take a bird's-eye view of activity in the separate fields of information retrieval and linguistics, and then consider the particular aspects of information retrieval which have an apparent linguistic content. The meat of the book comes in two chapters on syntax and semantics respectively, where a detailed analysis of the present state of linguistic theory is followed by a discussion of how this theory has been, or might be, applied to information retrieval. There is a further section on fact retrieval.

The authors lean fairly heavily on an analysis of the retrieval process which identifies three stages: informal interpretation of the document, formal representation of this interpretation, and manipulation of the representation in searching. One can imagine other views of the process (indeed, they mention another)—not necessarily better ones, but ones which emphasise other aspects. In some instances the authors are restricted by their particular approach. For example, they point out quite rightly that most comparative experiments that have been carried out with index descriptions using syntax seem to show that this syntax is not particularly useful in retrieval. But they do not consider one possibility: that the fact that the indexer (man or machine) has to formulate the description in a syntactically coherent form may influence his choice of terms, and hence may affect retrieval performance even if the terms are searched in a postcoordinate manner, ignoring syntax.

But this argument serves to reinforce one of the main conclusions of the book: that there is a lack of suitable theories of information retrieval which would indicate where linguistic theories might be of use. Overall, the conclusions are somewhat negative: both fields have to develop further before the one is likely to be a major contributor to the other. Indeed, there is some doubt that this will ever happen; but the authors argue

persuasively that there are still plenty of possibilities.

The book performs its function as a survey very well, with a surprisingly good international coverage. It is coherent and well thought out, and also well written. The production is decidedly cheap-looking (yellow paper, a number of misprints, badly aligned type and uneven type density). But in content the book is a valuable contribution to the literature, which should help to bring order to a somewhat fragmented field. S. E. ROBERTSON

Why plans go awry

Planning and Budgeting in Poor Countries. By Naiomi Caiden and Aaron Wildavsky. Pp. xvii+371. (Comparative Studies in Behavioural Science.) (Wiley: New York and London, March 1974.) £8.40.

A MAIN theme of this book had been anticipated by Bert Brecht in the *Threepenny Opera*. Freely translated, the song goes something like this:

Well, go and make a plan!

Seek where matrices lurk

Then go and make a second plan,

Neither of them will work.

Or, in the words of the Nepalese planner, summarising the experience of his country: "Try one form of organization; no result. Try something else; no result. The result is always the same" (Page 216).

The two authors of this book are political scientists who have covered more than 80 poor countries with questionnaires and interviews in order to examine government planning and budgeting and to recommend reforms. Their work reflects the disenchantment with planning.

Some of the criticisms of plans as ambitious, abstract, irrelevant and unrealistic miss the point. The purpose of formal models is to trace the indirect effects of alternative policies. Things often turn out differently in the second and third round from the intentions of the first. First-round socialism may turn into third-round monopoly capitalism. Particularly but not only in poor countries good causes tend to be turned into rackets. The reason why planning has failed, where it has failed, is not that it uses abstract models and traces interdependence between variables, but that it has sometimes been pseudo-planning, confined to a ceremonial superstructure, without being geared to where the action is.

The authors argue for the central importance of the annual budget. Though it is obviously true that no plan can be implemented unless it is integrated into the annual budget, there is a danger of mistaking a nec-

essary, though minor, condition for the strategic one. Budgets are, at best, annual public expenditure plans. A focus on fiscal magnitudes, though essential for proper public accounting, obscures and evades the real activities. Links between fiscal (or even financial) expenditures and results are tenuous, especially in underdeveloped countries. There are no fixed coefficients between money expenditure and land reform, population policy, incomes policy, education, public health, nutrition. Foreign exchange budgeting, manpower budgeting, raw material budgeting are just as important as fiscal budgeting and even they do not exhaust the range of necessary policies. Proper public accounting is necessary to ensure, negatively, that public money is not spent extravagantly or corruptly, but it cannot ensure, positively, that it is spent according to social priorities and that the necessary complementary actions are taken. Budgeting is to planning what bookkeeping is to business management: without it, management is impossible; but with the best book-keeper in the world, a firm can go bankrupt. These points are not enough stressed by the authors, who see the plan essentially as a many-year public capital budget.

Much is made of the need for redundancy. In a piece of exquisite jargon, the authors write: "Broadly speaking, we can regard social poverty as a lack of functional redundancy" (Page 49). But there is a vast difference between reserves (which serve a purpose) and redundancies. A more analytical and quantitative approach would have made the distinction clear. It is now well known that in poor societies, not only unskilled labour, but also capital and technically trained professional manpower are redundant: but, alas, they are not reserves.

The authors make a large number of entirely fair and commonsensical criticisms of planning. "If we were asked to design a mechanism for decisions to maximise every known disability and minimise any possible advantage of poor countries, we could hardly do better than comprehensive, multi-sectoral planning" (Page 293). The need for unavailable information, for political stability, for consistent aims are cited as unattainable conditions. But perhaps the most serious criticism of planning is omitted, viz. that its very success, measured by coherence and consistency, becomes an obstacle to adaptation and innovation. Plans introduce an additional rigidity into societies already inflexible. Plans, for this reason, in spite of their declared intentions, are elements strengthening conservatism.

The conclusion is not, however, reliance on *laissez-faire* and the free play

of market forces. The authors rightly point to the need for a combination of contingency planning, continuous budgeting and rolling planning, so that there can be adequate and speedy responses and adaptations to unforeseen events, both favourable and unfavourable.

The authors treat planners and planning as part of the social and political environment which they are supposed to plan. Planning the planners is not an invitation to an infinite regress but a reminder that there must be continuous mutual adaptation between plan objectives and social constraints.

PAUL STREETEN

Steroid receptors

Steroid-Cell Interactions. By R. J. B. King and W. I. P. Mainwaring. Pp. 440. (Butterworth: London, February 1974.) £10.

JENSEN and Jacobsen showed in 1962 that tissues such as the uterus and vagina, which are responsive to oestrogens, will retain administered oestradiol to a greater extent than non-responsive tissues and that this retention was due to the presence in the responsive tissues of specific receptor proteins. Since then the receptor hypothesis has been extended to many other hormones and the interaction between hormone and receptor is considered to be one of the links in the chain of events by which the hormone exerts its biological action. This concept has thus become of interest to molecular biologists as well as endocrinologists. The increased interest in this topic over the past five to six years has led to its discussion in numerous reviews and symposia; so what are the advantages of reading this monograph over consulting the reviews? Undoubtedly in the chapters reviewing the interaction of the various steroid hormones with the cell receptors there is more detail than found in most of the reviews. By far the main advantage, however, is the account of both the theoretical and practical background to the topic contained in the first two chapters on "Physicochemical Considerations of Steroid-Receptor Interactions" and "Methods Used to Study Steroid-Tissue Receptor Interactions" and the chapter giving a readable account of the molecular biological aspects of steroid-receptor interaction.

The title is slightly misleading since the monograph is mainly concerned with interactions involving receptors. Both androgens and oestrogens affect tissues or metabolic processes in which receptors have not yet been shown to be involved and this might suggest that there are other kinds of steroid-cell interactions. Many examples are

quoted by the authors. Androgens are anabolic but androgen receptors in skeletal muscle are difficult to identify nor has 5 α -reductase activity been demonstrated in the muscle of many species. Androgens stimulate both RNA and protein synthesis in liver but androgen receptors have not been identified in this tissue although it seems to contain oestrogen receptors. Normal breast tissue, which is influenced by oestrogens, accumulates oestradiol but does not seem to contain oestrogen receptors whereas breast tumours do. Little attention is paid to the interaction between the hormones in the tissues (uterus for example seems to contain an androgen receptor in addition to oestrogen and progesterone receptors), nor is the effect of other modifying influences on the hormones considered. This might be particularly important regarding events in the pituitary and brain. Conversion of testosterone to dihydrotestosterone seems not to be necessary for all biological activities of testosterone. Indeed some biological effects of testosterone seem to be produced by its conversion to oestrogen whereas dihydrotestosterone is not aromatised in this way. Until more knowledge is available it might be better to draw a distinction between androgen-sensitive tissues and androgen-dependent ones.

The authors point out the remarkable similarity of the different receptor proteins from different tissues. These similarities are deduced from current physicochemical data and it will be of interest to see whether they are upheld on further examination. Evidence that hormone binding correlates with hormone activity is reviewed. In respect of oestrogens and progesterone the evidence is quite good even though knowledge is limited. For androgens the situation is complicated by the extensive metabolism undergone by testosterone in the target organs and the possibility that the various metabolites may have different biological activities.

The monograph is well arranged and the newcomer to the field will have no difficulty in finding what he wants. In an attempt to bridge the gap between the writing of the book and its publication a summary of current literature is included. Whereas some of the chapters contain general summaries which are useful, others do not. I feel that the brief chapter on clinical and immunological aspects of steroid binding is superfluous to the main theme of the book. It will be a handy reference book to all investigators in this field and because of the amount of time it will save, it should be greatly appreciated by research students starting work in the area.

K. FOTHERBY

Spoonful of saccharin

Sensory Processes: The New Psychophysics. By Lawrence E. Marks. Pp. x+334. (Academic: New York and London, February 1974.) \$17.50; £8.25.

PEOPLE who use saccharin to sweeten their tea may have noticed a surprising thing: halving the concentration of sugar in a solution reduces its sweetness far more than halving an equally sweet (although much weaker) concentration of saccharin. This is one of many examples that demonstrate differing relationships between sensory magnitude and physical stimulus. How does one measure sweetness, brightness, pitch, odour? It has been shown, particularly in the pioneering work of the late S. S. Stevens, that asking subjects to assign numbers to sensation strength leads to a power function with an exponent dependent upon the stimulus and sensation considered. Lawrence Marks draws a clear distinction between this, the "new" psychophysics, and sensory physics, the "old" psychophysics, in which the observer is simply a detector of threshold, masked threshold, or null point, with measured quantities all in the physical domain. He describes and attempts to interrelate the various psychophysical procedures such as fractionation, category rating, and magnitude estimation and discusses the influences of extraneous factors. The senses are each considered under the headings of sensitivity, temporal and spatial factors, and qualitative aspects. Although one detects a certain antipathy towards the "old" psychophysics it is a carefully reasoned and comprehensive account and shows great concern for validation of the approach. Unfortunately his rather detached attitude coupled with the large number of references makes difficult reading in parts and a certain amount of repetition is inherent in the organisation he has adopted.

Although the new psychophysics gives insights into sensory processes not obtainable in any other way the field has a certain contrived air to it. We do not generally use our senses, or numbers, in this way. We normally use our sensory systems to perceive objects and relationships in the outside world. The idea that perceptions are built up from elementary sensations has been superseded by the concept of perception as an active, generative, process. We frequently see more than our sense organs convey because of past experience and expectations which can be triggered by a few salient features of sensory data. In this wider, more complex field of perception, detection could be of greater importance than sensory magnitude.

J. P. WILSON

Bouncing molecules

Chemical Applications of Molecular Beam Scattering. By M. A. Fluendy and K. P. Lawley. Pp. xi+400. (Studies in Chemical Physics.) (Chapman and Hall: London; distributed in the USA by Halsted Press, 1973.) £8.

THIS book is to be welcomed as the first non-specialist account of a challenging new field of chemical physics: the direct investigation of binary collision processes by the molecular beam technique. The authors discuss the design, performance and interpretation of the experiments, and also give an outline discussion of the background theory. The coverage reflects the present development of the field with major emphasis on elastic scattering and reactive processes.

The strongest chapters are those which deal with the technical design of the apparatus and with the analysis of elastic scattering experiments. Of these the sections of molecular beam sources, energy and state selection and detection and measurement will be of interest to a wide range of experimentalists. Readers with a more theoretical bias will be attracted by the clear account of quantal interference phenomena, seen not as abstract effects, but as sources of additional information in establishing the relation between the elastic scattering cross-sections and the intermolecular force field. The discussion of reactive scattering is equally complete in relation to present understanding, but the intrusion of mathematical detail makes it less easy to follow a clearly defined line of argument. Nevertheless there are few such comprehensive introductions in the literature. I only wish that the bibliography, which covers the important experimental work published before May 1972, could have been extended to include more recent studies on non-alkali atom systems, and that the tables could have contained a fuller resumé of available experimental information.

The level is that of the graduate student, since few undergraduates will have the necessary mathematical background. As such the book will be a welcome addition to the shelves of every serious chemical physics library.

M. S. CHILD

A cool look at doom

Man's Responsibility for Nature: Ecological Problems and Western Traditions. By John Passmore. Pp. x+213. (Duckworth: London, April 1974.) £5.95 cloth; £1.95 paper.

THE so-called 'environmental crisis' has been treated by scientists, economists and theologians, at levels ranging from

clinical objectivity to crude polemics; and it has been exploited by journalists and television producers. It is now the turn of philosophers; Professor Passmore's book is doubtless the first of a shelf-load of philosophical reflections on doom. It sets a very high standard in erudition, accuracy and clarity. The book opens with a masterly summary of the history of man's attitude to nature. Passmore is slightly apologetic about including this; he assures any anti-historical readers that they can, if they wish, skip it and go straight on to the second part of the book, dealing with ecological problems. In fact the historical essay is the best part of the book, and the only part with anything new to say. It describes vividly the Judaeo-Christian attitude to man, which divides him from other living things and makes him unique by investing him with a soul. This anthropocentric tradition has been blamed for man's exploitation of nature and it has prompted some writers to suggest that Western societies will not show proper concern for the environment unless they adopt a new ethic or a new religion, perhaps borrowed from the East.

Passmore argues powerfully and persuasively against this attitude, and especially against the implication that a scientific and rational attitude to nature cannot be reconciled with concern for the environment. The business of science, he says, is to turn mysteries into problems; only then can one set about trying to solve them. It was Descartes and Bacon, not the author of *Genesis*, who propagated the view that man can do as he pleases to nature with impunity. But there is no guidance to be found in the views of some contemporary theologians either; Passmore rejects Bishop Montefiore's idea of stewardship and Teilhard de Chardin's idea of cooperation with nature. He has no use for the arcadian musings of *Blueprint for Survival*. He realises that intellectuals like himself would be the first people to suffer if society were to discard some of the conveniences of modern technological society, such as air travel and motor cars.

From his historical summary Passmore turns to the contemporary ecological problems, which he distinguishes from "problems in ecology": the latter are scientific; the former are social. He covers familiar ground, reminding his readers (though it is superfluous for anyone likely to read this book) that ecological problems have three components: scientific, economic and ethical; and he brings the reader to the familiar conclusion: that the solution to ecological problems must be politically feasible. It is at this point that

the reflections of a philosopher might be expected to illuminate the issues. Unfortunately (but not surprisingly) Passmore's lucid analysis, like that of so many other writers, comes to a stop. We know that some ecological problems (such as pollution) can be solved within the framework of Western democratic institutions. We suspect that others (such as the dedication to ever-increasing material consumption) cannot. Before political decisions can be made an ethical question has to be answered: What duty, if any, do we have to posterity? If we cannot make sacrifices to ameliorate the human condition in the third world, which we have seen, is it likely that we shall make sacrifices to ameliorate the human condition of our great-grandchildren, whom most of us will never see?

Passmore seems to have a vague and tentative belief that this ethical question is soluble by rational thought in the framework of Western democratic institutions; perhaps by acting with concern for our offspring and expecting that they will act with equal concern for their offspring. It is a very modest conclusion to a very sophisticated essay. But Passmore is right to be modest. Heroics and utopias are the opiate of environmentalists. We can hope to proceed only through what William Blake called "Minute Particulars"

E. ASHBY

Defects in oxides

Oxide Semiconductors. By Z. M. Jarzebski. Translated from the Polish by B. Gryzbowska-Swierkosz. Translation edited by Brian Randall Pamplin. Pp. xi+285. (Pergamon: Oxford and New York; Wydawnictwa Naukowo-Techniczne; Warsaw, January 1974.) £6.

THIS is a translation of a treatise by a member of the Warsaw Research Centre for crystals and its title is misleadingly broad. It is written by an oxide physicist for oxide physicists and concentrates on the transport of carriers in these materials as influenced by defects. It certainly does not attempt to teach, although an early chapter, on preparation techniques for oxides, provides a useful survey of one aspect of oxide technology. The main interest of the author is the effect on the conductivity of oxides produced by heating them at various pressures of oxygen; the last half of the book is a survey of such effects in about ten metal oxides. The data on carrier transport are exhaustively reviewed but the physical mechanisms involved in this transport are referred to in a rather passing manner and certainly not treated at any depth.

The physics of the structure of defects in the oxides is avoided in a

manner which might almost be called studied, as if the author held that it was quite enough to note the entrance and exit of an electron or hole from the oxide without worrying very much about what happened to it while in this medium, except to note an activation energy for the process. This extends even to the chapters on zinc oxide, aluminium oxide and silicon dioxide, for which, in fact, a rich literature on defect structure exists. Other subjects which one would expect to be treated in a book of this title are carrier trapping (no mention at all); band and hopping conduction models (glancing references only); the role of the *d* orbitals in defect and band structure in the transition metals (orbitals mentioned only occasionally in passing); and the applications of oxide semiconductors (not even a bibliographical reference given). A subject which is treated well in the explanatory chapters is the law of mass action applied to interactions between oxide and oxygen at high temperatures, in which defects are treated as an active participant in the chemical reaction.

There seems somehow to be a gulf between the kind of defect science described here and the world of 'frozen-in' defects typical of radiation damage and mechanical deformation; the latter science uses a wide range of spectroscopic and scattering measurements to build structural and wave-mechanical pictures of defects, while the former relies largely on measurements of the transport of electrons or atoms in the solid. This book, while it can be read without difficulty, perhaps even with profit, by one of the 'frozen-in' community, does not bridge that gulf. Moreover, those who wish to use this book as a quick summary of the field should note that the references do not, except for a few cases, extend past 1970.

A. G. HOLMES-SIEDLE

The chemical industry

The Chemical Economy: A Guide to the Technology and Economics of the Chemical Industry. By B. G. Reuben and M. L. Burstall. Pp. xix + 530. (Longman: London, February 1974.) £6.95.

THAT chemistry courses must become more relevant has been the anguished cry of students and a source of frustration for many teachers of that subject for the past decade. Drs Burstall and Reuben have demonstrated what sort of effort is required if a beginning is to be made. They have written a long book crammed full of information directly related to their title. The book is well organised and the data in it are clearly presented so that prospective

readers are guided step by step through the complexities of chemical technology without undue fatigue. The primary objective of the book is to answer the question "How do we put chemistry to work for us?". As a second objective they intend to do something, in the concrete, to improve the spirit of university-industry cooperation. By providing such a lively and contemporary account of the ways in which chemical technology is used by the industry, the authors have achieved both of them. The book is aimed at an audience of first year university students having an A-level qualification in chemistry but no previous training in economics is presupposed.

This work is not an introductory course in chemistry juxtaposed with an elementary introduction to economics. Quite the contrary. The greater part of the book discusses a wide range of chemical reactions and processes but sight is never lost of the socioeconomic setting in which they function. By describing petroleum, natural gas, heavy organic chemicals, polymers, soaps and detergents, dyes and pigments, pharmaceuticals and heavy chemicals in relation to the firms, predominantly those in the United Kingdom, which utilise them, one is led, albeit in a piecemeal manner, to build up a picture of the chemical industry. A further section attempts to set the UK chemical industry into the world-wide complex of chemical industries. A disappointing feature of the presentation—though it would be difficult to remedy—concerns the historical description of the factors that have led up to the application of a particular process. These descriptions tend to be rather too brief and, consequently, may give the impression that chemical technology develops largely from discoveries in chemistry which appear, somewhat fortuitously, a little while before they are actually needed. None the less for students, who presumably know very little about this sector of industrial activity, it is perhaps a reasonable compromise to ensure that at least a little history is included with the technical and economic factors in the description of the industry.

The book also explores, in a descriptive manner, the economics of the firm. The aspects of microeconomics which are discussed include the decision of management to invest money in a new process and a very interesting section entitled "How do you know you are making money?". In a further chapter the authors discuss some of the problems of scaling up a laboratory experiment to industrial production. This leads to a discussion of the problems of disposing of the wastes from these processes and thence to some of the problems associated with pollution by in-

dustrial effluents.

The authors have produced a book which is doubly useful. It will certainly be useful for students of the growing number of broad-based science courses who are demanding that their curricula be relevant both to the society they live in and the sorts of jobs they may expect. For them it will provide more than a set of introductory materials because the authors have so organised the book that by following up the many references and footnotes, the student will be able to approach more closely the core of many contemporary technical and social problems. Also for the academic who is being asked to prepare more socially relevant course material this book should provide a useful guideline.

MICHAEL GIBBONS

Computer techniques

Operating Systems. By D. C. Tsichritzis and Philip A. Bernstein. Pp. xviii + 298. (Computer Science and Applied Mathematics: a Series of Monographs and Textbooks.) (Academic: New York and London, March 1974.) \$13.50; £6.36.

OPERATING systems research seems to have become a popular line with computer scientists, perhaps from a desire to put their own house in order before dealing with more general techniques. Most books about it fall into a middle range, assuming basic knowledge and providing abstracts of the more subtle techniques described in journals.

The authors of this book are clearly competent in their subject, so it is unfortunate that the book is not more readable. The volume is best described as loosely written, and the authors themselves felt compelled to apologise (page 121) for the incoherence of the first five chapters. In particular, the frequent cross references embedded in the text are particularly difficult to follow. Footnotes would have been much easier to scan, and would have helped maintain a continuity within the main text. The entire work carries an air of editorial neglect, and falls into the trap of collaborative work, repetition. Although the book starts at a low level, it would be very difficult for a student to follow.

Apart from the problems at the end of each chapter, and the admirable bibliography, the most worthwhile chapters cover design and implementation principles, dealing with problems of project management plus a collection of the authors' solutions. The book could be worth buying for these chapters alone; in fact the width of scope leaves me wondering why they did not publish three well organised volumes, instead of trying to cram the entire subject into 300 pages.

JEFFREY GRIBBIN

"It's like this . . ."

An exhibition of 500 years of pictures for science at Nottingham Castle, until September 15th.

Not so much an exhibition of pictures 'for science' as an exhibition of illustrations designed to make apparent that which is not immediately so, and with a rather heavy emphasis on anatomy and mechanics at the expense of 'pure' science notions. The devices which illustrators have employed in order to reveal the unseen realities of man's world, range from the simple stripping of skin from muscle and skeleton, to the cross-sectional representation beloved of Victorian steam engineers, on to the scanning electron microscope, whose shaded and textured images have so captured the lay reader's curiosity as to merit page spreads in the Sunday supplements on purely aesthetic grounds. Alas, no 200,000 times magnified study of the human sweat gland in sight here, but the bristle of a caterpillar does service to amaze the casual visitor.

The aim of the exhibition is not so much to illustrate the history of science as to make familiar some of the more visually striking works done for it. According to the organisers the visual interest of the pictures is firstly in the information which they were made to convey, but in the best of them this is raised to a 'celebration of understanding'—a rather attenuated concept to keep in mind when browsing over Robinson's engine working gear or Davis's Fire Escape Machine, regardless of the sturdy commonsense that these undertakings embodied. The impression one gathers from the narrative explaining the Fire Escape Machine is rather one of a mind awakening from slumber, and peering unsteadily at the ordering of things, than of a *bona fide* celebration of understanding.

"On the alarm of fire," John Davis remarked, circa 1810, "I would have the machine brought out directly, as I consider it an improvident method when a house has been on fire some time to have to search about for the keys of the church yard or some other obscure place, to bring the fire ladders . . ."

The celebratory aspect of the science illustrator's work is most plainly evident in the anatomical woodcuts and engravings of the centuries preceding the invention of photography. From the mediaeval autopsy studies of Mondino de' Luzzi, who dissected corpses in cases of suspected foul play, to Joseph Maclise's plates from the Victorian *Surgical Anatomy*, there is a distinct sense that apart from providing maps and tools for surgeons, the artists were revelling in the insights which their probing of cadavers had revealed. The

unravelling arrangement by Govaert Bidloo and Gerard de Lairese of the ligaments whose contractions control the movement of the human hand, for example, is presented with an objectivity which might equally have been applied to a study of a marionette's limb. And thereby the point is made: that the workings of human machinery are as much a function of mechanical and chemical laws as that of everything else in the universe—not an entirely uncontroversial proposition half way through the seventeenth century.

Apart from the insights which raise many of these studies to the level of fine art, one is aware of a scrupulous technical achievement in their execution and their conventions. The study on this week's cover, by Albinus and Wandelaar, is one of six plates derived from the *Tabulae Skeleti et Musculorum Corporis Humani* of 1747, a brilliantly engraved series, employing devastatingly complex perspective techniques devised by a professor of physics to ensure accuracy and uniformity. Albinus' own account of the process offers an understanding of the niceties involved:

"Not a single figure" wrote Albinus, "has been drawn free hand. All have been measured and brought down to scale, either from an indeterminate distance, as the architects do, a method which has been followed in most cases, or from a distance of 40 feet through diopters which correct to an indeterminate distance in such cases as, for instance, the pictures of skeletons, upon which finally, as upon a ground plan, the muscles have been drawn in. The tiny bones of the ear the artist measured with a very small and perfect compass." A system of nets in front of the subject allowed the artist to relate details to the whole in scale when he approached from his 40 feet vantage point to draw details. Most important of all, he drew nothing "he had not first thoroughly understood".

The portion dealing with modern science pictures, called 'Images of the Invisible', loses a little through familiarity: everybody knows from Arthur Mee's encyclopaedia what the drop of milk looks like when splashing into a saucer, and the bullet when hitting the shutter trip-wire. And to a lesser extent the zebra striped schlieren photographs of pressure waves caused by a body moving in a density stratified fluid, and field ion micrographs have lost their power to surprise anybody but the most unobservant man on the Clapham omnibus. The best loved exhibits in the show are camera obscuras which afford views of the city, a reflex camera obscura, which affords one the opportunity of drawing one's travelling companion, and a machine which affords sound the chance to be seen by using its varying frequencies to agitate the

surface of a pool of oil. All in all, a worthwhile undertaking which succeeds on its own modest terms.

JOHN HALL

Verdict on DDT

DDT has been accused of killing birds and fish, causing cancer, poisoning babies at the breast and disrupting the ecosystem of the oceans. Tuesday's Documentary, "The Rise and Fall of DDT," found the insecticide guilty on the first charge but innocent of all others except the last where a verdict of 'not proven' was reached. In mitigation it was shown beyond all reasonable doubt that DDT has saved the lives of millions of people throughout the world who would otherwise have died from malaria, typhus and other insect-borne diseases. With commendable objectivity the programme did not recommend a sentence.

The case of dichlorodiphenyltrichloroethane (DDT) is certainly a curious one and the producers of the documentary exploited this to their advantage. Although it is now a symbol of all things wrong with intensive farming, DDT was shown to begin life as a revolutionary breakthrough in pest control.

Old newsreel film skillfully illustrated DDT's first really big success in the Second World War. Shortly after the liberation of Naples, it was used there to forestall an epidemic of typhus—the first time that this had been done on a large scale. DDT was also shown to have played a vital part in the campaign against the Japanese. But the recipe for DDT is very simple and soon after the war it was being produced in bulk by various companies. This led to indiscriminate use; indeed DDT became so popular that it was made into an American cocktail—a 'Micky Slim'.

The documentary traced the beginnings of DDT's downfall to 1949 and the use of a similar compound, DDD, against mosquitos in Clear Lake, California. By 1954 the population of a rare grebe on the lake had dropped alarmingly and DDD was blamed. Studies on DDT in other environments followed and lawsuits naming the chemical began to be won. The change in public opinion was so great that even when President Nixon's environmental Protection Agency exonerated DDT, its findings were overruled and the insecticide banned from the United States.

Following this largely historical introduction, Tuesday's Documentary brought forward a long series of scientific witnesses to testify to the damage, or otherwise, that DDT inflicts on the environment. Throughout this argument, the programme success-

fully maintained its impartial attitude, described by one of its producers as "beleaguered neutrality".

It is just as well it did for a good deal of the evidence is contradictory. DDT was said to circulate in the air and soil, ultimately contaminating the sea but Dr George Harvey of Woods Hole Oceanographic Institute, Massachusetts, described his failure to find it there. When DDT was seen to distend the livers of mice it was said to lower their tolerance to some chemicals like carbon tetrachloride but actually increase their resistance to others such as aflatoxin. Almost all the evidence in the programme pointed to the sublethal effect of DDT but there was little opposition to the charges that

it was responsible for deaths due to eggshell thinness in birds and increased mortality in fish eggs.

Out of 23 factories which once produced DDT in the United States only one, Montrose Chemicals, survives. Its workers are continuously exposed to a high level of DDT and if there is any truth in the charge that DDT is carcinogenic then it should be found here. But the programme showed that these workers actually have a lower incidence of cancer than normal. On the dangers to human beings perhaps the most pertinent comment came from Professor James Busvine of the London School of Hygiene and Tropical Medicine who felt that DDT has never been proved to cause the death of a single

person but has undoubtedly saved up to ten million lives.

Just before the end of the film the increasing resistance of many insects to DDT was briefly mentioned. Nature itself seems to be passing the final judgment on DDT as more and more pests become immune to its effects. As the future of many underdeveloped countries depends on effective pest control and the use of DDT and other insecticides is becoming increasingly unsatisfactory (even leading to an increase of the pest in some cases), insect resistance is surely important enough to warrant more than this cursory treatment in an otherwise informative and balanced production.

JOHN WILSON

Announcements

Appointments

Anthony Kelly has been appointed Vice-Chancellor of the **University of Surrey**.

A. J. Leadbetter has been appointed to the chair of physical chemistry at the **University of Exeter**.

M. A. Sleight has been appointed to the chair of biology at the **University of Bristol**.

International meetings

September 19–20, **551st Meeting of the Biochemical Society**, University of St Andrews (The Meetings Officer, The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP).

September 24, **The Use of Molten Salts in Surface Treatment**, Leatherhead (Dr A. J. B. Cutler, C.E.R.L., Kelvin Avenue, Leatherhead, Surrey, UK).

September 24–25, **552nd Meeting of the Biochemical Society**, University College, Galway (The Meetings Officer, The Biochemical Society, 7 Warwick Court, Holborn, London WC1R 5DP).

September 25–26, **Symposium on the Chemistry of Liquid Metals**, University of Leicester (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

October 3, **Symposium on Micro Organisms in Food**, University of Leeds (Mr A. J. Crowther, The Metal Box Co. Ltd., 24 Fennel Street, Manchester M4 3FH, UK).

October 6–9, **6th Lunteren Lectures on Molecular Genetics**, Netherlands (Dr W. P. M. Hoekstra, Department of Microbiology, State University of Utrecht, Padualaan 8, Utrecht, The Netherlands).

October 7–12, **22nd International Meeting of Transportations and Communications**, Genoa (Segretaria Generale I.I.C., Villa Piaggio, Via Pertinace, 16125 Genoa, Italy).

October 10–12, **Genetic Engineering: Danger or Hope**, Davos (Rudolf Brun, Gottlieb Duttweiler Institute for Economic and Social Studies, CH-8803 Rüschlikon, Switzerland).

October 24, **Inaugural Meeting of the Hydrogeological Group of the Geological Society of London**, London (Dr J. D. Mather, Geological Society of London, Burlington House, London W1V 0JU).

Errata

In the article "Memory in enzyme membranes" by A. Naparstek *et al.* (*Nature*, **249**, 490; 1974) the legend to Fig. 1 should read . . . internal pH as a function of decreasing (a) and increasing (b), the external pH values.

In the article "Bioassay of a *Drosophila* pheromone influencing sexual selection" by J. E. Leonard, L. Ehrman and M. Schorsch (*Nature*, **250**, 261; 1974) the blocks for Figs 1 and 2 were reversed.

Reports and Publications

Great Britain

Science Research Council. The Work of the Rutherford Laboratory in 1973. Pp. 215 (Chilton, Didcot: Rutherford Laboratory, 1974). [246]
Standing Stones of Maeshowe of Stenness. By Magnus

Spence. Pp. 25. (London: Research into Lost Knowledge Organization, Mrs. Janette Jackson, 36 College Court, Queen Caroline Street, Hammersmith, 1974.) [246]

CIRCA—International Centre for Co-operation in Agricultural Research. Intensive Agriculture and the Environment. (North Western European Region Symposium, School of Agriculture, The University, Newcastle-upon-Tyne, 19–21st September 1973.) Pp. iv + 123. (Dublin: An Foras Taluntais, 1974.) £2. [266]

National Radiological Protection Board. NRPB-R17: The Determination of Plutonium in Urine by Ultrafiltration. By G. N. Stradling, D. S. Popplewell and G. J. Ham. Pp. 12. NRPB-R18: Measurement of Activity of Surfaces Contaminated by Electron-capture Nuclides. By W. J. Iles and D. F. White. Pp. 43. (Harwell, Didcot: National Radiological Protection Board, 1973 and 1974.) [266]

Oundle School Natural History Society Annual Report for 1973. Pp. 56. (Oundle, Peterborough: Oundle School Natural History Society, 1974.) [266]

Rothamsted Experimental Station. Report for 1973. Part 1: Pp. 412. Part 2: Pp. 276. (Lawes Agricultural Trust.) (Harpenden, Herts: Rothamsted Experimental Station, 1974.) £3 the two parts. [266]

Other countries

National Research Council of Canada. NRC Associate Committee on Scientific Criteria for Environmental Quality. Picloram: The Effects of Its Use as a Herbicide on Environmental Quality. (Subcommittee Report No. 1.) Pp. 128. (Ottawa: Publications, NRC, 1974.) [216]

National Institutes of Health, Bethesda, Md. Annual Report of International Activities, Fiscal Year 1973. Pp. vi + 100. (DHEW Publication No. (NIH) 74-374). (Bethesda, Md.: National Institutes of Health, 1974. For sale by US Government Printing Office.) \$1.35. [216]

Journal of Cutaneous Pathology. Vol. 1 No. 1, February, 1974. Edited by Leopoldo F. Montes. Pp. 71. Subscriptions: Vol. 1 (6 issues) D.kr. 220.00 plus postage D.kr. 14.00 (US \$38.60, £15.40, DM109.50) subject to exchange rate fluctuation. (Copenhagen, Denmark: Munksgaard, 1974.) [216]

Smithsonian Contributions to Zoology, No. 154: *Atenius*, *Aphotenius*, and *Pseudatenius* of the United States and Canada (Coleoptera: Scarabaeidae: Aphodiinae). By Oscar L. Cartwright. Pp. iv + 106. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$1.85 [216]

Smithsonian Contributions to Zoology, No. 169: Studies of Neotropical Caddisflies, XVIII: New Species of Rhyacophilidae and Glossosomatidae (Trichoptera). By Oliver S. Flint, Jr. Pp. iv + 30. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 80 cents. [216]

Environmental Conservation. Vol. 1 No. 1. 1974. Edited by Nicholas Polunin. Pp. 80. Subscriptions: Vol. 1 (Quarterly) SFRs 120 (approx. US \$41) (Lausanne, Switzerland: The Foundation for Environmental Conservation, 1974.) [216]

Smithsonian Contributions to Paleobiology, No. 20: Ultrastructural Studies on Graptolites, I: The Periderm and Its Derivatives in the Dendroidea and in *Mastigograptus*. By Adam Urbaneck and Kenneth M. Towle. Pp. iii + 48. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$1.35 [216]

Smithsonian Contributions to Zoology, No. 171: Spider Mites from Northwestern and North Central Mexico (Acarina: Tetranychidae). By D. M. Tuttle, E. W. Baker and M. Abbatiello. Pp. 18. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 60 cents. [216]